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Spray Drying of Inca Peanut Meal Protein Hydrolysate to Produce Protein Powder Drink Mix

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Abstract

The optimal conditions (maltodextrin concentration and inlet air temperature) for spray drying Inca peanut meal protein hydrolysate (IMPH) were determined. The maltodextrin concentration was varied at 5, 10 and 15 % by weight, and inlet air temperature was varied at 150, 160 and 170 °C. The IMPH was subjected to spray drying, where the feed solution was atomized into fine droplets and exposed to a hot air stream, leading to rapid drying and the formation of powder. The moisture, protein, yield, water activity and water solubility index (WSI) of IMPH powder were analyzed. The optimal condition for spray drying IMPH powder utilized 10 % maltodextrin and an inlet air temperature of 160 °C. Under these conditions, the moisture, protein, yield and WSI of IMPH powder were 2.93, 36.93, 38.95 and 99.59 %, respectively, while water activity was 0.41. IMPH powder particles were spherical and non-agglomerate. Inca peanut meal protein powder drink mix (IMPD) with cocoa flavor was the most acceptable. The IMPD with cocoa flavor could be promoted as a high protein and high dietary fiber product. It could be kept at 35 ± 2 °C for 12 weeks, whilst retaining good microbiological properties.

Keywords: Inca peanut meal, Spray drying, Maltodextrin, Protein drink mix, Protein hydrolysate

Introduction

Currently, the global obesity issue represents a huge challenge to public health, with its prevalence increasing despite rising awareness of the related health concerns and preventive actions. According to the latest World Health Organization (WHO) data, obesity rates have nearly tripled since 1975, impacting over 2 billion individuals globally, with over 650 million categorized as obese [1]. Obesity and metabolic diseases among young adults are increasing, and this population group is more interested in their health and weight control [2]. Weight control methods for most young adults involve consuming an alternative protein diet, drinking water instead of sugary drinks and taking diet pills [3]. An alternative-protein plant-based diet is popular among overweight young adults, wishing to control their weight [4]. High-protein diets have demonstrated their effectiveness in reducing body weight and enhancing body composition. They achieve this by reducing fat mass while preserving fat-free mass (FFM), regardless of whether individuals are following low-calorie or standard-calorie diets. Additionally, high-protein diets have shown promise in

preventing weight regain after initial weight loss, and no adverse effects on bone density or renal function in healthy adults have been reported [5]. Given the growing emphasis on healthy eating, especially in the context of fitness and weight management, high-protein diets have gained increasing attention. Thus, functional drinks are gaining more and more attention at present as these groups of young adults desire a nutritious, easy-to-drink product to effectively manage weight.

Functional drinks consist of nutrients such as amino acids, vitamins and minerals, as well as prebiotic blends to increase dietary fiber that help in excretion or the addition of protein alternatives to repair the body. A 2020 - 2021 survey of young adults in Thailand found that 83 % of consumers were willing to pay for plant-protein alternatives. Protein-alternative products derived from plants only constitute 11 % of the beverage market [6]. In South-East Asia in general, and in Thailand in particular, the consumption of plant-based beverages as part of the daily diet is the norm [3]. In the past, protein products were mainly consumed by bodybuilders and athletes but currently more people are consuming these products due to the rise of an interest in a healthy lifestyle [6]. Therefore, developing an accessible protein drink mix would be an interesting goal to respond to the needs of consumers. Potential plant alternatives include Inca peanut seed because of its high protein, high dietary fiber and high essential fatty acid and mineral content. Thus, it makes a good choice for beverage production.

Plukenetia volubilis, commonly known as Inca peanut, is a plant of the Euphorbiaceae family. Other names are Sacha inchi, Inca inchi and Mountain peanut, and it is widely used as a raw material in the edible oil industry [7]. This plant consists of essential oils (35 to 60 %) and its protein content is around 27 %. Inca peanut seed contains high amounts of essential amino acids such as histidine, leucine, lysine and tryptophan [8]. Inca peanut meal is a by-product of the oil extraction from Inca peanut seed using the cold pressing process. The high-pressure compression of the seeds occurs without heat. This method provides the highest quality and safety for consumers compared with using chemical solvents to process the expeller-pressed seeds. The obtained Inca peanut meal bio-active compounds still contain varying amounts of healthful ingredients including fatty acids (5.68 % unsaturated fat, 2.65 % omega 3 and 2.44 % omega 6), dietary fiber (11.06 %), essential amino acids (9.33 % histidine, 7.22 % leucine, 17.85 % lysine and 3.28 % tryptophan) and protein fragments (56.61 %) [7]. In recent years, the global market for Inca peanuts has seen a significant rise in popularity. This surge in demand has led to the introduction of various nutritional supplements, including gourmet oil, protein powder and encapsulated oil. Additionally, there are now options such as roasted, salted and candied Inca peanut seeds. Within the food industry, there is currently a growing interest in Inca peanut meal protein due to its appealing attributes, which include its functional properties, affordability, widespread availability, renewable nature and exceptional nutritional value [9].

However, high protein foods that contain beans and cereals can have adverse effects on health as each person reacts to protein differently. Some people can consume more protein without affecting their digestive system, while other people may experience bloating, diarrhea, and a feeling of incomplete bowel movement after ingesting more protein. This occurs because most legumes contain relatively high amounts of soluble oligosaccharides such as raffinose and stachyose and are broken down by bacterial fermentation in the intestine [10]. Other limitations regarding plant-based protein include antinutrient factors in some raw materials, which can adversely affect protein digestibility, absorption of amino acids and the quality of dietary protein [11]. Enzymatic hydrolysis of plant proteins has been applied to improved solubility compared to unmodified proteins. Hydrolytic treatment, including enzymatic hydrolysis, has shown positive effects on the foaming performance of plant proteins. It disrupts the compact tertiary structure of native proteins, generating smaller peptides that have faster diffusion and increased adsorption rates at the interface, facilitating foaming [12]. Enzymatic-hydrolysis of protein can remove hyper-allergic or anti-nutritional factors such as trypsin inhibitors, glycinin, β-conglycinin, phytate, oligosaccharides raffinose

and stachyose and saponins in soybeans. The protein hydrolysate can also reduce problems such as nut allergies, irritable gastrointestinal symptoms and the presence of bloody mucus in stools. In addition, protein hydrolysate is a protein molecule cleaved into smaller peptides or free amino acids, which are readily absorbed. Therefore, protein hydrolysate production is used in protein drink mixes obtained from plants [13].

Furthermore, the spray drying process has resulted in the production of a functional powdered beverage while preserving its physicochemical and nutritional attributes, including protein content [14]. Utilizing spray drying in beverages also offers several benefits, such as prolonging shelf life, enhancing convenience for both consumption and transportation, and inhibiting the growth of microorganisms. Raw materials for drying must be liquid or solution. In addition, increasing the solid content of the liquid feed by more than 10 to 50 % can reduce the energy used for drying by approximately 50 %. Spray drying is the process of spraying an aerosol liquid solution into highly heated air in a drying chamber, causing water to be rapidly extracted from the solute until the product becomes a concentrated solution or powder. Based on the research findings from various studies, the optimal inlet temperature for producing plant-based protein drink mix through spray drying varies depending on the specific protein source. In this study, the inlet temperature for the spray drying process started at 150 °C. Based on the research findings from various studies on spray drying of plant-based proteins, an inlet temperature of 150 °C has been shown to be beneficial for different types of protein powders. For instance, in the study on jack bean tempeh protein isolate found that an inlet temperature of 150 °C led to favorable moisture content and protein content values, indicating its suitability for protein isolates [15]. Moreover, the study on whey powder production highlighted that an inlet air temperature of 150 - 210 °C influenced various properties such as moisture content, bulk density and outlet air temperature, showcasing the importance of this temperature range in spray drying processes [16]. Therefore, based on the collective evidence, an inlet temperature of 150 °C appears to be a suitable choice for starter inlet temperature in plant-based protein spray drying processes**.** The addition of maltodextrin allows the food to be dried better in the drying process, reduces adhesion problems and results in higher yields [17]. This is because hydrolyzed starch derivatives such as maltodextrin are non-sticky products. Through the spray drying process, no clumping occurs. Therefore, this research aimed to study the optimal conditions and physico-chemical properties of the maltodextrin content and inlet temperature for formula development of protein drink mix products.

Materials and methods

Preparation of Inca peanut meal (IPM)

IPM was obtained from Inca peanut seeds (*Plukenetia volubilis)* harvested in Chiang Rai Province, Thailand, via oil extraction (Nikao Corporation Co., Ltd.). The material was boiled at 100 °C for 20 min, then filtered using 4 layers of straining cloth and dried in a hot air oven at 60 °C for 8 h. The treated meal was milled (SG-400-03, Dxfill Machine, China). After that, the IPM extraction was defatted by hexane (Food grade 30 to 50 %, STN Chemical Co., Ltd. Thailand) with a ratio of IPM to hexane of 1:3 (w/v) at room temperature for 24 h, where the first 5 h involved continuous stirring. Defatting was performed twice until the final oil content was below 1 %. The hexane residues in IPM were analyzed using the IUPAC Method 2.607. After drying in a tray dryer at 60 °C for 2 h, the IPM was ground and sieved using an 80-mesh wire screen and stored in plastic bags laminated with aluminum foil, vacuum-sealed and kept in freezer at −20 °C until used.

Preparation of IPM protein hydrolysates (IMPH)

IPM flour (1 g) was dispersed in distilled water (DW, 10 mL). DW was sterilized using an autoclave at 121 °C for 1 h and 1 N HCl was used to adjust the pH to 6.0 of the samples. Then samples were incubated at 50 °C for 10 min for efficiency before initiating the reaction of enzymatic hydrolysis. The bromelain enzyme was adjusted to pH 7 with 1 N NaOH. The enzyme concentration was 20 % (w/v), and hydrolysis time 360 min. The hydrolysis reaction was run in a flask using a stirred water bath at 50 °C and neutral pH. Then the enzyme was stopped by heating to 95 °C for 15 min. The protein hydrolysate was centrifuged at 8,000 rpm and 4 °C for 30 min and then filtered through filter paper (Whatman No. 1) to separate the supernatant of IMPH. The IMPH produced by bromelain enzyme was stored in brown glass bottles at −20 °C before spray drying.

Drying process of IMPH

The IMPH was mixed with maltodextrin (DE-10, Chemipan Corporation Co., Ltd, Bangkok, Thailand) by homogenizer. The maltodextrin concentration was varied at 5, 10 and 15 % by weight. The mini spray dryer [18] used an airbrush nozzle cap and the speed of raw material feed flux was 0.5 mL and 5 mL/min, respectively. Inlet air temperature varied (150, 160 and 170 °C). The obtained powders were gathered in a cyclone and stored in laminated plastic bags. The IMPH powder was analyzed for physicochemical properties such as color values (L*, a* and b*), total soluble solid, water activity, moisture, protein, yield and solubility.

Physicochemical properties of IMPH powder

1) Color value

The IMPH powder filled approximately 1/4 of the glass. The sample was spread as a smooth surface and the color values (L*, a* and b*) were measured by colorimeter (Hunter Lab ColorFlex EZ, Hunter Associates Laboratory, Inc. USA). The lightness (L*) ranged from 0 (lightness) to 100 (darkness), redness (a*) range from redness (+) to greenness (–) and yellowness (b*).

2) Total soluble solid

Total soluble solid (°Brix) was measured by hand refractometer (PAL-1 Atago, Japan)

3) Water activity (a_w)

The IMPH powder filled approximately 1/3 of the sample cup. The sample was spread as a smooth surface and water activity was measured by a_w meter (Novasina, The Art of Precision Measurement, Inc. Switzerland) and read at room temperature.

4) Moisture content

Moisture was analyzed by AOAC (2016). The IMPH powder (2 g) was contained in an aluminum cup (the aluminum cup was dried and weighed before using). The sample was dried in a hot air oven at 100 °C for 1 h, and then placed in a desiccator for 30 min. After that, the sample was weighed and the moisture content was calculated.

5) Protein content

The protein content was analyzed using the Kjeldahl method [19].

6) Yield

The percentage yield of IMPH powder was calculated from the weight of IMPH powder and weight of IMPH solution before drying following the formula below [18].

% Yield = (IMPH powder/weight of solution before drying)×100.

7) Water solubility index (WSI)

The WSI of IMPH powder was determined using IMPH powder (1 g) and 10 mL of water placed in a microcentrifuge tube at 30 °C. The resulting sample was centrifuged at 3,000 rpm for 10 min. The supernatant was poured into an aluminum cup and dried in a hot air oven at 105 °C for 24 h [20]. Percentage WSI was calculated:

% WSI = (dry weight of supernatant/dry weight of IMPH powder)×100.

Scanning electron microscopy

Scanning electron microscopy (SEM) (QuantaTM 250 FEG, Fédération Équestre Internationale, Switzerland) was used to evaluate the morphology of IMPH powder particles. Samples were detected using an accelerating voltage of 5 kV at 10.0 mm (WD = 10.0 mm). Sample micrographs were represented by a 10 μm scale.

Development of protein drink mix from IMPH powder

The optimal condition of IMPH powder was selected by analyzing the best physicochemical properties. The IMPH powder was used to produce IMPD. The IMPD formular was modified from Queiroz *et al.* [21]. The ingredients of IMPD were composed of 22 % IMPH powder, 20 % IPM, 31 % sugar, 13 % creamer and 14 % cocoa flavor (**Table 1**).

Table 1 Formular of IMPD with cocoa flavor.

Ingredients	(g/100 g)
IMPH powder	22
IPM	20
Sugar	31
Creamer	13
Cocoa flavor	14

The optimal ratio between inulin and sugar

The IMPD formular (**Table 1**) had varying ratios of inulin (Orafti®GR inulin 90 %, BENEO. EU) and sugar at 50:50, 60:40, 70:30 and 80:20 by weight (**Table 2**). Other ingredients were kept constant. The IMPD sample (50 g) was kept in laminated plastic bags (size 10×15 cm^2) for analyzing physicochemical and sensory properties.

Table 2 Ratio between inulin and sugar in the protein drink mix powder.

Treatment*	Inulin:Sugar
1	50:50
2	60:40
3	70:30
4	80:20

*Treatment (50 g) included 11 g IMPH powder, 10 g IPM, 15.5 g sugar, 6.5 g creamer and 14 g cocoa powder.

1) Color value

The color values (L*, a* and b*) of IMPD were measured.

2) Dietary fiber

The dietary fiber (Fructans: Inulin + Fructo-oligosaccharide) was analyzed by an in-house method based on AOAC (2016) 997.08.

3) Sensory evaluation: The IMPD sample (4 g) was kept in plastic laminated bags with a zip lock (size 10×8 cm^2) for sensory evaluation. Samples were labelled with a 3 digit-code. The IMPD was dissolved in hot water (~ 90 °C) and the ratio of IMPD: Water was 4:20 w/v. Sensory evaluation applied a 9-point hedonic scale (9 = like extremely, 1 = dislike extremely) for color, aroma, taste, viscosity and overall acceptability. Sweetness was evaluated using a 5-point scale (5 = too much, 1 = too little). The sensory properties of the IMPD drink were evaluated by 50 panelists aged between 20 to 39 years. Panelists were staff or students of the Department of Nutrition, Faculty of Public Health, Mahidol University, Thailand.

Optimal content of flaxseed in protein drink mix

The IMPD with the optimal ratio of sugar and inulin was varied flaxseed at 1, 2, 4 and 8 % by weight. Other ingredients were kept constant, except for cocoa powder which was changed following the percentage of flaxseed. The IMPD sample (50 g) was stored in laminated plastic bags with zip locks (size 10×15 cm^2) until analysis of physicochemical and sensory properties.

1) Color value

The color values (L*, a* and b*) of IMPD were measured.

2) Omega 3

The omega 3 fatty acid content of IMPD with flaxseed at 1, 2, 4 and 8 % was determined by gas chromatography (Agilent 7890, Agilent Technologies, Wilmington, DE, USA.) according to the AOAC method [19]. Sample extraction was achieved using a Soxhlet extractor and ether solvent. The obtained oil was stored in Eppendorf tubes and the air was removed and replaced with nitrogen. The omega 3 fatty acid was identified by comparing the retention times to the known standards. The results were expressed as g fatty acid/100 g sample.

3) Viscosity

The viscosity of IMPD drink with different ratios of flaxseed were measured using a viscometer (Viscometer DV3TTM, AMETEK Brookfield, Inc. USA). Spindle no.1 with a speed range of 65 to 250 rpm and shear rate of 79 to 305 s^{-1} were used to determine viscosity, and the sample temperature was controlled while viscosity was measured at 25 °C and reported in centipoises (cP).

4) Sensory evaluation

The sensory properties (except sweetness) of the IMPD drink were measured.

Selection of the flavoring agent for IMPD

There were 4 flavors of IMPD: Cocoa, green tea, Thai tea and coffee. Other ingredients were kept constant except for the cocoa powder which was changed following the determination of flavors. The IMPD sample (50 g) was kept in laminated plastic bags (size 10×15 cm^2) to analyze physicochemical and sensory properties.

1) Sensory evaluation

The sensory properties (except sweetness) of the IMPD drink were measured.

2) Total phenolic content (TPC) and antioxidant activities

TPC: The TPC of IMPD was ascertained using an antioxidant assay based on electron transfer (Folin-Ciocalteu method) as adjusted by Amarowicz *et al.* [22]. IMPD (25 µL) and 50 µL of 10 % Folin-Ciocalteu

reagent were pipetted in to 96-well microplates. After 5 min, 7.5 % sodium bicarbonate (200 µL) was added and incubated in a dark room at room temperature for 120 min. The value of absorbance was measured using a microplate reader (Synergy HT, Bio-Tex Instruments, VT, USA) at 765 nm. Preparation standards using the gallic acid concentration were 10, 20, 40, 60, 80, 100 and 200 ug/mL, respectively. A blank used deionized water as the standard. The results were reported as mg gallic acid equivalent (GAE) per 100 g sample. This experiment was tested in triplicate.

Antioxidant activities: DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) free radical method. DPPH method radical scavenging activities (1,1-diphenyl-2-picrylhydrazyl) were ascertained following the adjusted method of Kanlayavattanakul *et al.* [23]. IMPD (22 µL) was mixed with DPPH solution (150 µM of DPPH in 200 µL of 95 % ethanol) and contained in 96 well plates. After that, the samples were incubated in a dark room at room temperature for 30 min and then the value of absorbance at 530 nm was measured by microplate reader. Preparation standards using Trolox concentration were 0.01, 0.02, 0.04, 0.08, 0.16, 0.32 and 0.64 mM, respectively. A blank of the standard solutions comprised of 95 % ethanol. The results were reported as µM Trolox equivalent per 100 g sample.

Ferric reducing antioxidant power assay (FRAP assay): The FRAP assay was adjusted using the method of Benzie *et al.* [24]. Each IMPD sample (22 µL) was mixed with 150 µL of FRAP reagent, placed in 96 well plates and incubated in a dark room at room temperature for 8 min. The value of absorbance was measured by microplate reader at 595 nm. Preparation standards using Trolox concentration were 7.8125, 15.625, 31.25, 62.5, 125 and 250 µM, respectively. A blank of the standard solutions comprised of deionized water. The results were reported as µM Trolox equivalent per 100 g sample.

The oxygen radical absorbance capacity (ORAC assay): The ORAC assay was adjusted using the method of Ou *et al.* [25]. The IMPD (25 µL) samples were mixed with 30 µM of fluorescence solution and placed in black plate 96-well plates and incubated at 37 °C for 15 min. After that, 2,2'-azobis (2-amidinopropane) dihydrochloride (AAPH) was monitored for 90 min using a microplate reader with an excitation wavelength of 485 nm and an emission wavelength of 528 nm. Preparation standards using Trolox concentration were 3.125, 6.25, 12.5, 25, 50 and 100 µM, respectively. A blank of the standard solutions comprised of ORAC buffer. The final ORAC values were calculated using the differences of area under the fluorescence decay curve (AUC) between the blank and the sample. The area under the decay curve (AUC) was calculated using the following equation:

$$AUC = (0.5 + f5/f4 + f6/f4 + f7/f4 + \ldots + fi/f4) \times CT$$

where $f4$ = initial fluorescence reading at cycle I; CT = cycle time in minutes; The results were reported as micromole Trolox equivalent per 100 g sample of dry weight.

Nutritional value

Chemical composition

The moisture, protein, fat, ash, crude fiber and carbohydrate contents of the best IMPD formular were determined using AOAC methods (2016). Carbohydrate content was calculated by difference (100 − % moisture − % protein − % fat − % ash − % crude fiber protein, fat, ash, crude fiber).

Shelf life of IMPD with cocoa flavor

The IMPD with cocoa flavor was studied regarding the length of time for which an item remains usable and fit for consumption. The sample was contained in laminated zip lock plastic bags (size 12×18 cm^2) 50 g of IMPD for storage. The samples were stored separately in a hot air oven set at temperatures of

35, 45 and 55 °C and were only taken out when analysis was required. The physicochemical and sensory properties of the IMPD with cocoa flavor were tested after storage for 0, 3, 6, 9 and 12 weeks.

Color value

The color values (L*, a* and b*) of IMPD with cocoa flavor at 35, 45 and 55 °C were measured using a colorimeter (Hunter Lab ColorFlex EZ, Hunter Associates Laboratory, Inc. USA). The lightness (L*) ranged from 0 (lightness) to 100 (darkness), redness (a*) ranged from redness (+) to greenness (–) and yellowness (b*).

Viscosity

IMPD with cocoa flavor at 35, 45 and 55 °C (50 g) were mixed with water (250 mL) and its viscosity were measured.

Moisture content

The moisture content of IMPD with cocoa flavor at 35, 45 and 55 °C were measured.

Water activity (a_w)

The a_w of IMPD with cocoa flavor at 35, 45 and 55 °C were measured.

Lipid oxidation using TBARS

The thiobarbituric acid reactive substances (TBARS) values were colorimetrically determined [26]. Therefore, 1 g of IMPD with cocoa flavor at 35, 45 and 55 °C were measured in triplicate, inside a 15 mL falcon tube and mixed with a 5 mL solution containing 7.5 % trichloroacetic acid solution (TCA), 0.1 % propyl gallate and 0.1 % EDTA-Na2, along with 5 mL of TBA reagent (0.02 M thiobarbituric acid in distilled water). The tubes were incubated at 100 °C for 40 min and cooled to ambient room temperature in an ice bath. Once cooled, the absorbance of samples were measured at 532 and 600 nm.

Microbiological quality

The microbiological qualities of IMPD were determined at 35, 45 and 55 °C. IMPD with cocoa flavor was tested for Bacillus cereus according to ISO 7932: 2004, Clostridium perfringens according to ISO 7937: 2004 and Staphylococcus aureus according to ISO 6888-1: 1999/Amd 2: 2018. Total plate counts were determined according to the FDA-BAM [25]. Yeast and mold counts were determined according to the AOAC International [19]. The data were reported as CFU/g.

IMPD with cocoa flavor was tested for Salmonella spp. according to ISO 6579-1: 2017 and was reported per 25 g sample. Escherichia coli was tested according to the FDA-BAM method [27]. The data were reported as MPN/g.

Sensory evaluation

IMPD with cocoa flavor at 35, 45 and 55 °C (4 g) were kept in plastic, laminated zip lock bags (size 10×8 cm^2) for sensory evaluation (except sweetness attribute).

Shelf-life index

The Q10 method evaluation was modified from the method of Hitchins *et al.* [27] Q10 is the quotient between the reaction velocity at a determined temperature and a 10 °C higher temperature. The θs(T) is the storage time of IMPD with cocoa flavor at 45 °C (day), whereas θs(T + 10) is the storage time of IMPD

with cocoa flavor at 55 °C (day). The ΔT is the difference between storage temperature at 45 °C and prediction temperature at 35 °C (45 - 35 °C). The Qs(T) is the storage time of prediction temperature at 35 °C (day), while the Qs(T + 10) is the storage time of IMPD with cocoa flavor at 45 °C (day). The storage time of the IMPD with cocoa flavor at 35 °C (day) was calculated as follows:

$$Q10 = \theta s(T)/\theta s(T + 10)$$

$$Q1 = Q100.1$$

$$Q\Delta T = Qs(T)/Qs(T + 10)$$

Statistical analysis

Mean values, standard deviation and analysis of variance (ANOVA) were computed using a commercial statistical package SPSS, Version 18.0 (SPSS Inc., Chicago, IL, USA). These data were obtained from physicochemical properties and were investigated using 3 repetitions of a completely randomized design (CRD) experiment. The sensory evaluation employed a randomized completely block design (RCBD) for analysis. The variance and mean values were analyzed for difference using Duncan's multiple range tests at a 95 % significance level. Significant differences were regarded when $p \leq 0.05$.

Results and discussion

Production of IMPH powder by spray drying process

Effect of spray drying on physicochemical properties of IMPH

The maltodextrin concentration and inlet temperature influenced physicochemical properties (color value, total soluble solid, water activity, moisture, protein, yield and WSI of IMPH powder.

(1) The color value and total soluble solid

The lightness (L*), redness (a*) and yellowness (b*) values of IMPH ranged from 86.25 to 92.64, 0.10 to 1.75 and 8.09 to 24.28, respectively (**Table 3**). The color of IMPH powder appeared light yellow. The elevated amount of maltodextrin increased the lightness value, while the redness value was low because of the white color of maltodextrin. Paseephol and Jaengsanam [28] reported that adding more than 10 % maltodextrin resulted in reduced redness and increment in the lightness of betalain from celery. Conversely, the increase in inlet temperature had no significant effect on the color of IMPH powder. Statistical analysis indicated no significant differences in color values across varying temperatures, likely due to the rapid nature of the spray drying process, which minimizes impact on color. Similarly, Ahmed *et al.* [29] reported that maltodextrin-treated flours had considerably higher L* values than controls, possibly due to enhanced anthocyanin and total phenolic content. When compared to the control, a* values tended to decrease.

Before drying, total soluble solids of protein hydrolysate powder ranged from 6.02 to 6.45 °Brix. Nevertheless, increasing the maltodextrin content resulted in an increase in the total soluble solids from 10.27 to 16.50 °Brix as well as yield and protein content

Table 3 Effect of inlet air temperature and maltodextrin content on color values (L*, a* and b*) and total soluble solid of IMPH powder.

Inlet temperature (°C)	Maltodextrin (%)	Total soluble solids (°Brix)	Color values			Appearance
			L*	a*	b*	
150	5	10.27 ± 0.06^{c}	86.33 ± 0.02^{c}	1.75 ± 0.01^{a}	24.28 ± 0.02^{a}	
	10	14.13 ± 0.06^{b}	90.29 ± 0.03^{b}	0.15 ± 0.01^{b}	12.06 ± 0.02^{b}	
	15	16.50 ± 0.10^{a}	92.64 ± 0.01^{a}	0.12 ± 0.01^{c}	8.13 ± 0.01^{c}	
160	5	10.27 ± 0.06^{c}	86.25 ± 0.02^{d}	1.74 ± 0.02^{a}	24.19 ± 0.01^{a}	
	10	14.20 ± 0.05^{b}	90.19 ± 0.01^{b}	0.15 ± 0.01^{b}	12.05 ± 0.01^{b}	
	15	16.17 ± 0.12^{a}	92.60 ± 0.01^{a}	0.10 ± 0.03^{e}	8.09 ± 0.01^{d}	
170	5	10.30 ± 0.13^{c}	86.29 ± 0.01^{d}	1.74 ± 0.03^{a}	24.22 ± 0.01^{a}	
	10	14.20 ± 0.10^{b}	90.17 ± 0.02^{b}	0.15 ± 0.01^{b}	12.01 ± 0.02^{b}	
	15	16.27 ± 0.06^{a}	92.61 ± 0.03^{a}	0.11 ± 0.01^{d}	8.12 ± 0.01^{c}	

These results were expressed as mean ± standard deviation (SD) from triplicate determination. $^{a-e}$ Means followed by different letters within the same column indicate significant differences ($p \leq 0.05$).

Table 4 Effect of inlet air temperature and maltodextrin content on yield (%), protein (%), moisture (%), water activity and solubility (%) of IMPH powder.

Inlet temperature (°C)	Maltodextrin (%)	Yield (%)	Protein (%)	Moisture (%)	a_w	WSI (%)
150	5	10.48 ± 1.23^{e}	37.55 ± 0.50^{a}	4.84 ± 0.11^{a}	0.55 ± 0.51^{a}	96.69 ± 0.41^{c}
	10	38.52 ± 1.06^{c}	36.96 ± 0.63^{a}	4.58 ± 0.07^{b}	0.54 ± 0.18^{a}	96.71 ± 0.34^{c}
	15	41.66 ± 0.71^{b}	28.99 ± 0.71^{d}	4.46 ± 0.10^{b}	0.53 ± 0.01^{a}	96.73 ± 0.32^{c}
160	5	17.58 ± 1.25^{d}	37.43 ± 0.26^{a}	3.23 ± 0.05^{c}	0.47 ± 0.01^{b}	99.46 ± 0.33^{a}
	10	38.95 ± 1.66^{c}	36.93 ± 0.17^{a}	2.93 ± 0.15^{d}	0.41 ± 0.01^{b}	99.59 ± 0.02^{a}
	15	42.63 ± 0.35^{b}	28.34 ± 0.45^{d}	2.95 ± 0.06^{d}	0.39 ± 0.01^{b}	99.46 ± 0.11^{a}
170	5	19.24 ± 0.66^{d}	35.34 ± 1.22^{b}	2.39 ± 0.06^{e}	0.33 ± 0.02^{c}	97.45 ± 0.46^{b}
	10	39.62 ± 1.12^{c}	34.39 ± 0.57^{c}	2.34 ± 0.11^{e}	0.32 ± 0.01^{c}	97.18 ± 0.07^{b}
	15	45.63 ± 0.91^{a}	28.28 ± 0.41^{d}	2.37 ± 0.07^{e}	0.32 ± 0.02^{c}	97.48 ± 0.34^{b}

These results were expressed as mean ± standard deviation (SD) from triplicate determination. $^{a-e}$ Means followed by different letters within the same column indicate significant differences ($p \leq 0.05$).

2) Yield and protein content

The yield value of IMPH powder ranged from 10.48 to 45.63 % as shown in **Table 4**. Maltodextrin concentration and inlet temperature directly escalated yield value. Using an inlet temperature of 170 °C and adding 15 % maltodextrin when spray drying IMPH showed the highest value of IMPH powder yield (45.63 %) because the high inlet temperature drying resulted in protein hydrolysate powder with low moisture and water activity. Therefore, IMPH powder had a lower probability of residue in the spray dryer machine than using a low inlet temperature. The highest protein content ranged from 34.39 to 37.55 % indicating no statistical significance ($p > 0.05$) between maltodextrin concentration (5 to 10 %) and inlet temperature (150 to 160 °C). Maltodextrin concentration reduced the protein content in protein hydrolysate powder. The study found that increasing maltodextrin content improved yield but decreased protein content in IMPH

powder. At all tested temperatures (150, 160 and 170 °C), higher maltodextrin levels consistently resulted in higher yields and lower protein percentages. These findings suggest that optimizing maltodextrin levels can enhance production efficiency while balancing the nutritional profile of the final product. In addition, the inlet temperature significantly affected the protein content ($p \leq 0.05$), especially the inlet temperature of 170 °C. This inlet temperature could destroy protein structure as a result of the high inlet temperature drying process. Kruapongsak *et al.* [30] also reported that spray drying sericin powder at high temperatures resulted in the denaturation of globular sericin protein particles.

3) Moisture content and water activity (a_w)

Using a high inlet temperature and high maltodextrin concentration resulted in low moisture and water activity contents. This is because high inlet temperature causes evaporation of water faster in IMPH powder [28,31]. Moreover, maltodextrin treatment reduces the moisture absorption of spray-dried flours, making them less hygroscopic. This effect is due to maltodextrin acting as a carrier and coating agent [29]. Spray drying using an inlet temperature of 150 °C and adding 5 % maltodextrin resulted in a IMPH powder with a high moisture content (4.84 %) (**Table 4**). When the moisture in the powder particles is high, it agglomerates and decreases solubility [32]. The difference in maltodextrin content did not affect the moisture content of the IMPH powder ($p > 0.05$). This was because the high inlet temperature of spray drying resulted in higher evaporation of water in the IMPH powder than using a low inlet temperature. Wongsa-Ngasri *et al.* [33] found that the inlet temperature for spray drying protein hydrolysate from pickled jellyfish was 120 °C and added 2 % maltodextrin concentration. The highest moisture content of pickled jellyfish protein hydrolysate was 7.49 %, but using an inlet temperature of 180 °C and adding 6 % maltodextrin resulted in the lowest moisture content (2.09 %). However, IMPH powder using an inlet temperature of 170 °C produced small powder particles and a low moisture content. Thus, IMPH powder from this spray drying condition had poor water-solubility due to agglomeration and swelling. High quality IMPH powder should contain a water activity and moisture content of less than 0.6 and 6 %, respectively.

4) Water solubility index

WSI values of IMPH powder ranged from 96.69 to 99.59 % as shown in **Table 4**. Using an inlet temperature at 150 °C and adding 5 % maltodextrin showed the lowest WSI of IMPH powder because the powder from the spray drying process possessed a spherical shape and a wet surface surrounding. These characteristics of the individual powder particles cause mutual attraction until they clump together [30,34]. Using an inlet temperature of 160 °C and adding 10 % maltodextrin concentration resulted in the highest WSI of IMPH powder, due to the high temperature and wet surface surrounding the powder. The results of different maltodextrin concentrations did not affect the WSI ($p > 0.05$). Research findings from various studies provide insights into the differentiation of increasing maltodextrin concentration to 10 % with a spray drying inlet temperature of 160 °C. An experiment on spray-dried tomato powders revealed that higher inlet temperatures and maltodextrin concentrations, specifically at 10 % and 160 °C, resulted in better stability, with maltodextrin of 4 - 7 dextrose equivalents being the most effective carrier agent [35]. Similarly, a study on red spinach instant powder drink demonstrated that a 20 % maltodextrin concentration with a spray drying temperature of 160 °C produced the best product in terms of preference, yield and nutrient content [36]. Furthermore, optimizing spray drying conditions for nettle extract powder showed that increasing maltodextrin concentration and inlet air temperature improved process yield and encapsulation efficiency, while retaining functional properties of the extract [37]. These findings collectively suggest that increasing maltodextrin concentration to 10 % with a 160 °C inlet temperature can enhance product quality and stability in spray drying processes. An inlet temperature of 170 °C was a very high drying temperature, resulting in significant differences in WSI at 150 and 160 °C ($p \leq 0.05$). This was

probably because using a very high inlet temperature resulted in small powder particles and an escalation of porosity. Therefore, these characteristics of the powder caused swelling until solubility reduced.
Khuenpet *et al.* [38] reported a reduction in WSI when using high inlet temperatures during spray drying. The best spray drying condition for IMPH was using an inlet temperature of 160 °C and adding 10 % maltodextrin. This condition showed the highest values of yield, protein content and WSI but the lowest values of water activity and moisture content.

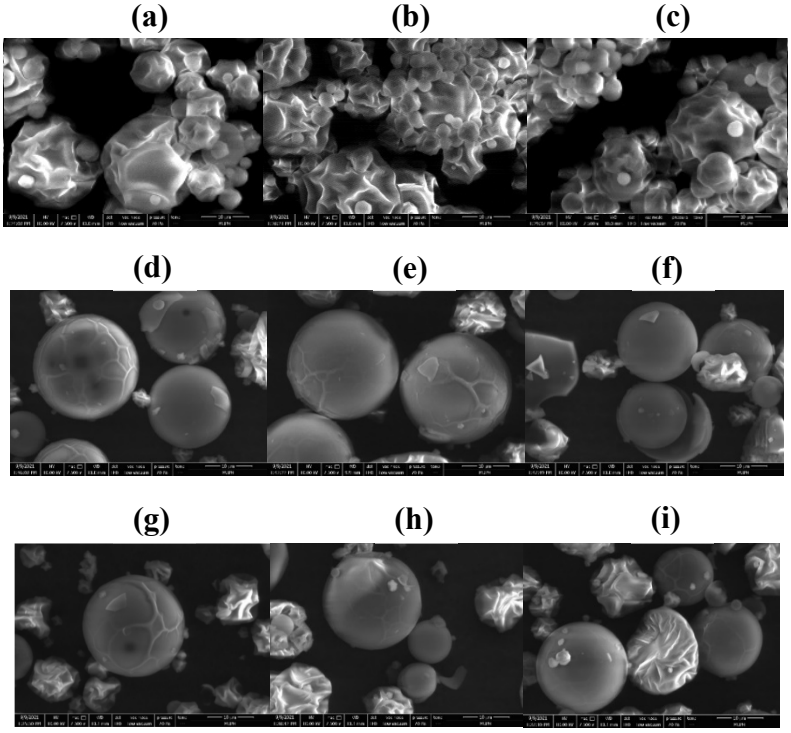

Figure 1 Particle sizes of IMPH powder from different inlet temperatures and maltodextrin contents at magnification of 7,500× (a) 150 °C, 5 % maltodextrin; (b) 150 °C, 10 % maltodextrin; (c) 150 °C, 15 % maltodextrin; (d) 160 °C, 5 % maltodextrin; (e) 160 °C, 10 % maltodextrin; (f) 160 °C, 15 % maltodextrin; (g) 170 °C, 5 % maltodextrin; (h) 170 °C, 10 % maltodextrin; (i) 170 °C, 15 % maltodextrin.

5) Microstructure and particle size of IMPH powder

The microstructure and particle size of IMPH powder at different inlet temperatures and maltodextrin concentrations using SEM micrographs are shown in **Figure 1**. IMPH powder which was produced using an inlet temperature of 150 °C and adding 5, 10 and 15 % maltodextrin had wrinkled and agglomerated particles, as indicated in **Figures 1(a)** - **1(c)**, respectively. This was probably because an inlet temperature of 150 °C created a high moisture content and the highest water activity and agglutination characteristics of the IMPH powder (**Table 4**). Moreover, the stickiness of the IMPH powder is related to the low glass transition temperature (Tg). Thus, when the IMPH solution was exposed to low drying temperatures, it became subjected to transformations, from a glass to a gummy state. On the other hand, the IMPH powder using an inlet temperature at 160 and 170 °C and adding 5, 10 and 15 % maltodextrin showed spherical and non-agglomerated powder particles. These results related to moisture content and water activity because the high spray drying temperature led to higher water evaporation and higher dispersion. Furthermore, an increase in powder is related to increasing inlet temperature. Protein hydrolysates contain low molecular weight peptides, are highly hygroscopic, and can either remain as a syrup or stick to the dryer chamber wall

during spray drying, causing wall deposition. Caking can also manifest during storage [39,40]. Karaca *et al.* [41] also investigated the use of maltodextrin as a carrier in the spray-drying process of sour cherry juice concentrate. The use of these carriers significantly affected the yield and glass transition temperature (Tg) of the resulting sour cherry powder. The authors also noted that the choice of maltodextrin as a carrier type influenced the yield, Tg, and total phenolic content of the spray-dried sour cherry powder. The addition of high molecular weight additives (carriers) such as maltodextrin to the solution can raise the overall Tg of the mixture, avoiding structural changes that affect the retention of volatiles in the powders during drying and /or storage. Tonon *et al.* [42] also confirmed that distinct inlet temperatures affect the surface smoothness of the particles. In addition, Tonon *et al.* [42]; Alamilla-Beltrán *et al.* [43]; Nijdam *et al.* [44] revealed that the average size of particles dried using a high inlet temperature was greater than at a low inlet temperature. Cai and Corke [34] confirmed that amaranthus betacyanin pigment particles remained fixed as large-sized globules during spray drying at high inlet temperatures. The material contained more water during the drying process at a higher inlet temperature as a result of the rapid rate of drying and the particles remaining fixed as globules.

Developing IMPD from IMPH powder

The optimal ratio between inulin and sugar in IMPD

The IMPD was studied the optimal ratio between inulin and sugar. The ratio of inulin to sugar varied at 50:50, 60:40, 70:30 and 80:20, respectively.

1) Sensory acceptance

From the sensory acceptance results shown in **Table 5**, the color values of 4 different IMPD ratios of inulin and sugar were not significantly different ($p > 0.05$). A ratio of inulin to sugar of 80:20 produced the highest dietary fiber content (14.26 %), followed by 70:30 (13.98 %), 60:40 (12.14 %) and 50:50 (11.93 %), respectively. However, a ratio of inulin to sugar of 60:40 was not significantly different from a 50:50 ratio ($p > 0.05$). The 50:50 ratio of inulin to sugar scored highest for appearance, color, aroma, taste, viscosity and overall acceptability (**Table 5**). The sweetness score of IMPD with a 50:50 inulin to sugar ratio was close to 3 (just about right), which was significantly different ($p \leq 0.05$). Thus, the optimal inulin to sugar ratio of 50:50 was selected to study the optimal flaxseed quantity for producing IMPD.

Table 5 Sensory acceptance scores of the protein drink mix containing different ratios of inulin and sugar.

Sensory acceptance scores	Inulin:Sugar			
	50:50	**60:40**	**70:30**	**80:20**
Appearance[1]	7.24 ± 0.92[a]	7.16 ± 0.98[a]	6.98 ± 1.42[ab]	7.06 ± 0.59[a]
Color[1]	7.66 ± 0.52[a]	7.78 ± 0.84[a]	7.58 ± 1.18[a]	7.21 ± 0.34[a]
Aroma[1]	7.74 ± 0.83[a]	7.18 ± 0.92[ab]	7.18 ± 0.84[ab]	6.92 ± 0.90[b]
Taste[1]	7.18 ± 0.69[a]	7.12 ± 0.56[a]	5.88 ± 1.35[b]	4.90 ± 0.84[c]
Viscosity[1]	7.34 ± 0.52[a]	7.06 ± 0.61[a]	4.52 ± 1.42[c]	6.12 ± 1.18[b]
Overall acceptability [1]	7.82 ± 0.77[a]	7.02 ± 0.89[b]	6.78 ± 1.23[bc]	6.20 ± 0.94[c]
Sweetness[2]	2.96 ± 0.19*	2.34 ± 0.24	2.12 ± 0.38	2.02 ± 0.55

[a-c] Means followed by different letters within the same row indicate significant differences ($p \leq 0.05$).

* The score of 3 (just-about-right score) indicates significant differences ($p \leq 0.05$).

[1] 9-point hedonic scale (9 = like extremely, 1 = dislike extremely).

[2] 5-point just about right scale (5 = too much, 1 = too little).

2) The optimal flaxseed content in IMPD

The physicochemical properties of IMPD containing different flaxseed powder contents are presented in **Table 6**. All the color values of 4 different flaxseed levels of the IMPD samples did not significantly differ ($p > 0.05$). The viscosity and omega 3 content of IMPD formulas that contained different levels of flaxseed powder were significantly different ($p \leq 0.05$). Adding 8 % flaxseed powder showed the highest IMPD viscosity. The omega 3 content in IMPD with 4 % flaxseed powder (127.94 mg/100 g) did not significantly differ from the IMPD with 8 % flaxseed powder (129.12 mg/100 g) ($p > 0.05$). The scores of appearances, color and aroma of IMPD containing different levels of flaxseed were not significantly different ($p > 0.05$) (**Table 7**). The IMPD with 4 % flaxseed powder showed the highest scores of tastes, viscosity and overall acceptability. Adding flaxseed powder aimed to increase omega 3 content and omega 6 and omega 3 ratio of the IMPD product. Diets high in omega 6 fatty acids and a high omega 6 to omega 3 ratio have been associated with weight gain in both animal and human studies, whereas a high omega 3 fatty acid diet decreases the risk of weight gain [45]. Therefore, the addition of 4 % flaxseed powder in the IMPD was used to study the optimal flavoring agent for use in the protein drink mix.

Table 6 Physicochemical properties of the protein drink mix containing different levels of flaxseed powder.

Physicochemical properties	Flaxseed content			
	1 %	**2 %**	**4 %**	**8 %**
L*^{ns}	71.65 ± 0.42	71.59 ± 0.44	71.28 ± 0.68	71.17 ± 1.24
a*^{ns}	5.22 ± 1.13	5.30 ± 1.61	5.34 ± 0.94	5.42 ± 0.78
b*^{ns}	11.02 ± 0.12	10.96 ± 1.02	10.73 ± 1.42	10.54 ± 0.38
Viscosity (cP)	2.95 ± 0.02^{d}	3.78 ± 0.01^{c}	8.42 ± 0.01^{b}	16.67 ± 0.01^{a}
Omega 3 (mg/100 g)	96.34 ± 0.01^{c}	103.45 ± 0.01^{b}	127.94 ± 0.01^{ab}	129.12 ± 0.01^{a}

These results were expressed as mean ± standard deviation (SD) from triplicate determination.
$^{a-d}$ Means followed by different letters within the same row indicate significant differences ($p \leq 0.05$).
^{ns} Not significant ($p > 0.05$).

Table 7 Sensory acceptance scores of the protein drink mix containing different levels of flaxseed powder.

Sensory acceptance scores	Flaxseed			
	1 %	**2 %**	**4 %**	**8 %**
Appearance ^{ns}	7.46 ± 1.2	7.64 ± 1.25	7.68 ± 0.52	7.66 ± 1.37
Color ^{ns}	7.87 ± 1.23	7.80 ± 1.12	7.92 ± 0.54	7.86 ± 1.35
Aroma ^{ns}	7.52 ± 1.23	7.62 ± 1.56	7.56 ± 0.27	7.68 ± 1.19
Taste	7.44 ± 1.34^{b}	6.72 ± 1.44^{c}	8.40 ± 0.34^{a}	6.96 ± 1.29^{c}
Viscosity	7.40 ± 1.11^{ab}	6.77 ± 0.16^{b}	8.08 ± 0.71^{a}	5.74 ± 1.16^{c}
Overall acceptability	7.82 ± 1.17^{ab}	7.32 ± 0.96^{b}	8.02 ± 0.14^{a}	6.32 ± 0.96^{c}

These results were expressed as mean ± standard deviation (SD) from triplicate determination.
$^{a-c}$ Means followed by different letters within the same row indicate significant differences ($p \leq 0.05$).
^{ns} Not significant ($p > 0.05$).

The optimal flavoring agent for IMPD

The appearances of IMPD with 4 flavors, i.e., cocoa, green tea, Thai tea and coffee are shown in **Figure 2**. The IMPD with cocoa flavor scored the highest on appearance, color aroma, taste, viscosity and overall acceptability (**Table 8**). The IMPD with cocoa and green tea flavors showed the highest TPC and antioxidant activities and did not significantly differ ($p > 0.05$) (**Table 9**). In addition, both flavors had TPC and antioxidant activity values that did not significantly differ ($p > 0.05$). The optimal flavoring agent for IMPD was cocoa because it showed the highest sensory scores for appearance, color, aroma, taste, viscosity and overall acceptability, and the highest TPC and antioxidant activities.

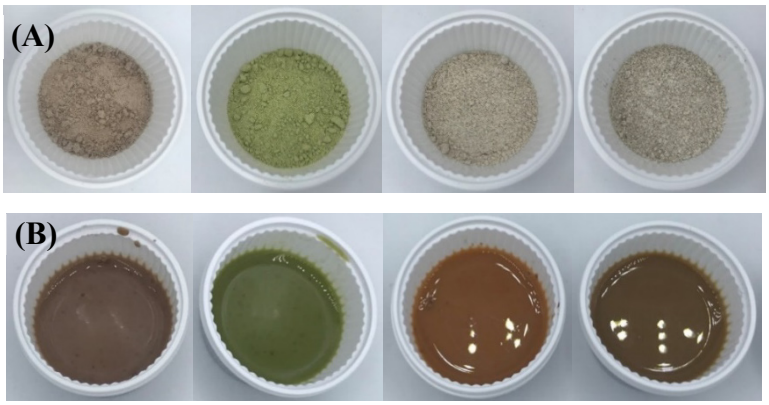

Figure 2 Appearance of IMPD with different flavors. (A) IMPD with cocoa flavor, green tea flavor, Thai tea flavor and coffee flavor (From left to right). (B) Ready-to-Drink IMPD with cocoa flavor, green tea flavor, Thai tea flavor and coffee flavor (From left to right).

Table 8 Sensory acceptance scores of IMPD with 4 flavors.

Sensory acceptance scores	Flavoring agents			
	Cocoa	**Green tea**	**Thai tea**	**Coffee**
Appearance	7.90 ± 0.65^{a}	7.56 ± 0.86^{b}	8.04 ± 0.35^{a}	7.18 ± 1.41^{b}
Color	8.32 ± 0.55^{a}	7.60 ± 0.90^{b}	8.16 ± 0.62^{a}	7.16 ± 1.11^{c}
Aroma	8.06 ± 0.47^{a}	7.44 ± 1.11^{b}	8.16 ± 0.65^{a}	6.58 ± 1.42^{c}
Taste	7.94 ± 0.37^{a}	7.40 ± 1.34^{b}	8.00 ± 0.61^{a}	6.70 ± 1.28^{c}
Viscosity	8.06 ± 0.79^{a}	7.60 ± 0.73^{b}	7.84 ± 0.65^{b}	6.98 ± 0.89^{c}
Overall acceptability	8.26 ± 0.44^{a}	7.90 ± 0.79^{b}	8.16 ± 0.46^{a}	7.22 ± 0.97^{c}

These results were expressed as mean ± standard deviation (SD) from triplicate determination. $^{a-c}$ Means followed by different letters within the same row indicate significant differences ($p \leq 0.05$).

Table 9 Total phenolic contents (TPC) and antioxidant activities of IMPD with 4 flavors.

Antioxidant values	Flavoring agents			
	Cocoa	**Green tea**	**Thai tea**	**Coffee**
TPC (mg GAE/g)	3.96 ± 1.74^{a}	4.03 ± 1.21^{a}	3.77 ± 0.48^{ab}	3.89 ± 1.14^{ab}
DPPH (µmol TE/ g)	14.06 ± 0.84^{ab}	15.25 ± 1.34^{a}	12.49 ± 1.03^{c}	6.57 ± 1.03^{d}
FRAP (µmol TE/g)	74.69 ± 1.28^{a}	63.28 ± 0.81^{b}	17.64 ± 1.19^{d}	24.21 ± 1.82^{c}
ORAC (µmol TE/100 g)	83.21 ± 1.56^{a}	83.57 ± 1.44^{a}	82.98 ± 1.18^{ab}	79.34 ± 0.28^{b}

These results were expressed as mean ± standard deviation (SD) from triplicate determination. $^{a-d}$ Means followed by different letters within the same row indicate significant differences ($p \leq 0.05$).

Nutritional value of IMPD with cocoa flavor

Nutritional composition

The total energy, carbohydrate, protein, fat, dietary fiber, ash and moisture contents per 50 g serving of IMPD with cocoa flavor were 160.72 kcal, 11.02 g, 19.71 g, 4.2 g, 10.81 g, 2.53 g and 1.73 g, respectively (**Table 10**). Moreover, one serving (50 g) of cocoa-flavored IMPD could be promoted as a high-protein-high-dietary-fiber- food product because it contains more than 50 % of the Thai Dietary Reference Intakes for protein and dietary fiber [46].

Table 10 Nutritional composition of the IMPD with cocoa flavor per serving (50 g).

Composition	IMPD with cocoa flavour
Total energy (kcal/50 g)	160.72
Carbohydrate (g/50 g)	11.02
Protein (g/50 g)	19.71
Fat (g/50 g)	4.2
Dietary fiber (g/50 g)	10.81
Ash (g/50 g)	2.53
Moisture (g/50 g)	1.73

Omega 3 fatty acid content

The IMPD with cocoa flavor also contained 128.80 mg of omega 3 fatty acid/100 g, which was approximately 50 % of the Thai DRI (250 to 650 mg/day) [41].

Shelf life of the IMPD with cocoa flavor

Fifty g of IMPD with cocoa flavor was packed in a zip lock laminated plastic bag. The IMPD with cocoa flavor was analyzed by determining physicochemical properties, microbiological quality and sensory acceptance after storing at 35, 45 and 55 °C for 12 weeks. The IMPD with cocoa flavor was taken out every 3 weeks for analysis.

Physicochemical properties

The physicochemical properties (color value, viscosity, moisture content, water activity (aw) and lipid oxidation using TBARS) of IMPD with cocoa flavor during storage were analyzed.

1) Color value

The color values of IMPD with cocoa flavor are exhibited in **Table 11**. Increasing storage time also increased the lightness (L*), redness (a*) and yellowness (b*) values of IMPD with cocoa flavor. Furthermore, storage time at 55 °C clearly changed at 3 to 12 weeks are shown in **Figure 3**. These changes usually occur over time as a function of increased storage temperature and relative humidity. The Maillard reactions are highly influenced by the a_w value because it has been generally accepted that the Maillard reaction rate is at its maximum around a_w 0.5 to 0.7. In addition, the Maillard reaction is responsible for nothing short of developing the IMPD, especially its sensory character. Tunick *et al.* [47] also reported that the whey protein powder became yellower by storing at 35 °C and was removed from the study after 12 months because of an unsatisfactory appearance. Rao and Labuza [48] reported on the effect of moisture content on the color change in egg white powders after 4 months storage at 23 °C. Notably, the amount of

residual glucose (reduced sugar) in egg white powder involved in the Maillard reaction, had a significant impact on product quality.

Table 11 Color values (L*, a* and b*), viscosity, moisture, water activity (a_w) and TBARS of the IMPD with cocoa flavor during storage at 35, 45 and 55 °C for 12 weeks.

Temperature (°C)	Storage time (week)	Color			Viscosity (cP)^{ns}	Moisture (%)	a_w	TBARS (mg MDA/kg)
		L*	a*	b*				
35	0	74.42 ± 1.32^{a}	4.06 ± 1.45^{e}	10.64 ± 1.07^{f}	8.41 ± 0.93	3.46 ± 0.12^{d}	0.274 ± 0.31^{ef}	0.32^{k}
	3	74.38 ± 1.67^{a}	4.05 ± 0.94^{e}	10.70 ± 1.23^{f}	8.49 ± 0.36	3.62 ± 1.03^{cd}	0.279 ± 1.22^{ef}	0.45^{j}
	6	70.28 ± 0.38^{c}	5.57 ± 1.92^{c}	10.89 ± 0.18^{f}	8.51 ± 1.31	3.88 ± 0.84^{c}	0.289 ± 0.44^{e}	0.59^{i}
	9	69.98 ± 1.17^{c}	5.53 ± 0.26^{c}	10.91 ± 1.18^{f}	8.58 ± 0.84	3.96 ± 0.22^{c}	0.303 ± 0.12^{e}	0.68^{h}
	12	64.52 ± 0.67^{de}	5.69 ± 1.04^{c}	11.25 ± 0.41^{e}	8.64 ± 1.06	4.05 ± 0.19^{c}	0.321 ± 0.12^{d}	0.77^{g}
45	0	74.53 ± 0.93^{a}	4.11 ± 1.12^{e}	10.26 ± 1.02^{f}	8.45 ± 1.12	3.43 ± 0.08^{d}	0.269 ± 0.15^{f}	0.31
	3	71.80 ± 1.33^{b}	5.01 ± 1.36^{d}	10.70 ± 0.93^{f}	8.68 ± 0.37	3.86 ± 1.23^{c}	0.293 ± 1.03^{e}	0.69^{h}
	6	69.96 ± 0.26^{c}	5.53 ± 0.04^{c}	10.68 ± 1.23^{f}	8.72 ± 1.44	4.08 ± 1.12^{c}	0.305 ± 1.22^{e}	0.82^{f}
	9	66.40 ± 0.89^{d}	6.64 ± 1.75^{b}	12.79 ± 1.03^{d}	8.81 ± 0.97	4.17 ± 0.92^{bc}	0.367 ± 0.23^{d}	0.98^{e}
	12	60.23 ± 1.46^{f}	6.85 ± 1.03^{b}	13.46 ± 0.72^{c}	8.83 ± 1.35	4.46 ± 1.01^{b}	0.396 ± 1.12^{c}	1.24^{d}
55	0	74.76 ± 0.88^{a}	4.10 ± 0.14^{e}	10.55 ± 0.31^{f}	8.39 ± 1.37	3.42 ± 0.25^{d}	0.273 ± 1.23^{ef}	0.30^{k}
	3	68.87 ± 1.48^{c}	5.76 ± 1.23^{c}	14.88 ± 0.17^{b}	8.52 ± 1.03	3.97 ± 1.34^{c}	0.319 ± 1.14^{d}	0.76^{g}
	6	65.85 ± 0.11^{d}	5.92 ± 1.02^{c}	15.03 ± 0.62^{ab}	8.71 ± 0.98	4.56 ± 1.27^{b}	0.427 ± 1.22^{c}	1.67^{c}
	9	63.59 ± 1.42^{e}	6.86 ± 0.38^{b}	15.26 ± 1.03^{a}	8.84 ± 1.04	5.28 ± 0.44^{ab}	0.538 ± 0.92^{b}	1.84^{b}
	12	61.17 ± 1.34^{ef}	8.49 ± 0.21^{a}	15.84 ± 1.95^{a}	8.86 ± 0.93	5.69 ± 1.81^{a}	0.744 ± 0.27^{a}	2.25^{a}

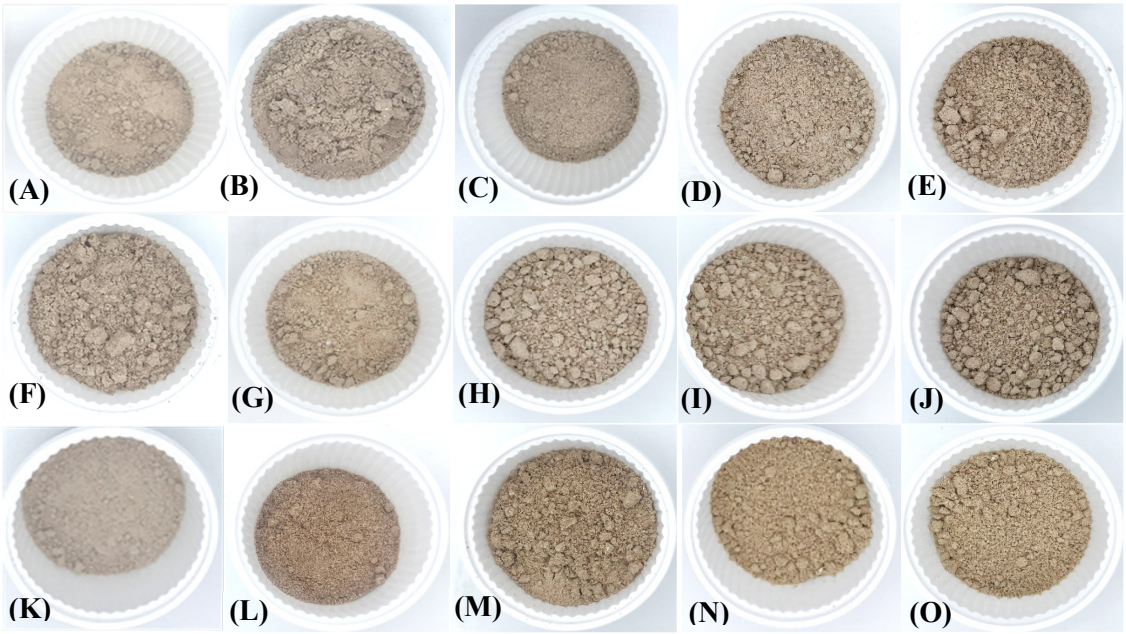

Figure 3 Appearance of IMPD with cocoa flavor during storage at (A) 35 °C for 0 week, (B) 35 °C for 3 weeks, (C) 35 °C for 6 weeks, (D) 35 °C for 9 week, (E) 35 °C for 12 weeks, (F) 45 °C for 0 week, (G) 45 °C for 3 weeks, (H) 45 °C for 6 weeks, (I) 45 °C for 9 weeks, (J) 45 °C for 12 weeks, (K) 55 °C for 0 week, (L) 55 °C for 3 weeks, (M) 55 °C for 6 weeks, (N) 55 °C for 9 weeks and (O) 55 °C for 12 weeks.

2) Viscosity

The viscosity value of the IMPD with cocoa flavor are presented in **Table 11**. The viscosity of IMPD with cocoa after storing at different conditions did not significantly differ ($p > 0.05$) and ranged from 8.39 to 8.86 cP (**Table 11**).

3) Moisture content and water activity (a_w)

The moisture content and water activity of cocoa-flavored IMPD stored at 3 different temperatures are shown in **Table 11**. The IMPD with cocoa flavor stored at 35, 45 and 55 °C for 0 to 12 weeks had moisture contents ranging from 3.46 to 4.05, 3.43 to 4.46 and 3.42 to 5.69 %, respectively. The a_w values of cocoa-flavored IMPD stored at 35, 45 and 55 °C for 0 to 12 weeks ranged from 0.274 to 0.321, 0.269 to 0.396 and 0.273 to 0.744, respectively. Storage time and temperature affected both moisture content and water activity. The a_w and moisture content of the IMPD with cocoa flavor stored at 35 °C for 0 to 12 weeks did not significantly differ ($p > 0.05$). On the other hand, the a_w and moisture content of this IMPD with cocoa flavor stored at 45 and 55 °C for 0 to 12 weeks significantly differed ($p \leq 0.05$). Consumer safety standards require that cocoa-flavored IMPD should have an a_w less than 0.6 and a moisture content less than 10 % [49]. These values resulted in microbial quality and safety for consumers. An a_w less than 0.6 can retard microbial growth. Thus, cocoa-flavored IMPD stored at 35 and 45 °C for 12 weeks could also retard microbial growth.

4) Lipid oxidation

The TBARS values of the IMPD with cocoa flavor stored at 35, 45 and 55 °C for 0 to 12 weeks ranged from 0.32 to 0.77 mg MDA/kg, 0.31 to 1.24 mg MDA/kg and 0.30 to 2.25 mg MDA/kg, respectively. Increasing storage time and storage temperature also increased TBARS values. In conclusion, the TBARS values of IMPD with cocoa flavor stored at 35 °C for 12 weeks were lower than those of the cocoa-flavored IMPD stored at 45 and 55 °C in the same week. Therefore, storage at 35 °C for 12 weeks showed the lowest lipid oxidation. In addition, high lipid oxidation resulted in product rancidity. TBARS values should not exceed 1.6 mg MDA/kg for general food because food remains acceptable to consumers and does not become rancid [50]. The shelf life of instant cereal beverage products showed that TBARS tended to be higher with longer shelf life. The initial TBARS value of the product was 0.74 mg MDA/kg for 10 weeks of storage. The storage of products at 35, 45 and 55 °C showed TBARS values of 7.33, 7.53 and 8.49 mg MDA/kg, respectively [51].

The microbiological quality of IMPD with cocoa flavor at week 0 is presented in **Table 12** and while stored at 35, 45 and 55 °C for 3, 6, 9 and 12 weeks is presented in **Table 13**. The total plate count, yeast and molds, Salmonella spp. Staphylococcus aureus, Bacillus cereus, Clostridium perfringens and Escherichia coli of IMPD with cocoa flavor at week 0 were in the limit of the specified notification of the Ministry of Public Health (No. 416) criteria. In addition, microbiological quality indicators were within the limits of the same notification after storage of the IMPD at 35, 45 and 55 °C for 3, 6, 9 and 12 weeks. The reason for this result might be because the IMPD with cocoa flavor was kept in effective packaging resulting in the product having a low moisture content and a_w throughout the storage period.

Table 12 Microbiological quality of IMPD with cocoa flavor at week 0.

Microbiological quality	IMPD	Guideline*
Total plate count (CFU/g)	< 10	1×10^4
Yeast and mold (CFU/g)	< 10	< 100
Salmonella spp. (per 25 g)	ND	ND
Bacillus cereus (CFU/g)	< 10	< 10
Clostridium perfringens (CFU/g)	< 10	1×10^3
Staphylococcus aureus (CFU/g)	< 10	1×10^3
Escherichia coli (per 1 g)	< 3	< 3

*The notification of the Ministry of Public Health (No.416) (Office of the Board and Ministry of Public Health, 1979). ND, not detected.

Table 13 Microbiological quality of IMPD with cocoa flavor during storage at 35, 45 and 55 °C for 3 to 12 weeks.

Temperature (°C)	Storage time (week)	Total plate count (CFU/g)	Yeasts and mold (CFU/g)
35	3	< 10	< 10
	6	< 10	< 10
	9	< 10	< 10
	12	< 10	< 10
45	3	121	< 10
	6	130	< 10
	9	139	< 10
	12	142	< 10
55	3	168	< 10
	6	180	< 10
	9	194	< 10
	12	206	< 10

Sensory acceptance

The sensory acceptance scores of the IMPD with cocoa flavor stored at 35, 45 and 55 °C for 0, 3, 6, 9 and 12 weeks are presented in **Table 14**. The viscosity values of this IMPD stored at the 3 temperatures for 12 weeks were not significantly different ($p > 0.05$). Likewise, the appearance, color, aroma, taste and overall acceptability scores when stored at 35 °C for 0 to 9 weeks (ranging score from 7.00 to 8.00) did not significantly differ ($p > 0.05$). On the other hand, the panelists did not accept the appearance, color, aroma, taste and overall acceptability of the IMPD with cocoa flavor stored at 55 °C for 9 to 12 weeks because of rancidity. However, these IMPD samples (except the sample stored at 55 °C for 9 to 12 weeks) were evaluated as acceptable (appearance, color, aroma, taste and overall acceptability scored greater than 4) and rancidity was unperceived. The overtime different sensory attributes decreased score, as well as at a higher storage temperature, more deterioration occurred [52].

Shelf-life index

The shelf-life index of the IMPD with cocoa flavor was evaluated using the Q10 technique. This method accelerates the temperature while stored to allow the product to deteriorate faster than usual. This method is used to estimate the effect of storage conditions on the product. Storage conditions at 45 and 55 °C were selected as the accelerated conditions. A TBARS value (above 1.6 mg MDA/kg) indicated the end of product shelf life because the product became rancid and unacceptable. An a_w more than 0.6 can increase microbial growth. The a_w and TBARS values of the IMPD with cocoa flavor stored at 45 °C were 0.598 and 1.63 MDA/kg, respectively, and its shelf life was 105 days. The a_w and TBARS value of the IMPD with cocoa flavor stored at 55 °C was 0.427 and 1.67 MDA/kg, respectively, and its shelf life was 42 days. The shelf-life estimation of the IMPD with cocoa flavor stored at 35 °C was 262.5 days, as calculated using the equation below.

$Q10 = \theta s(45\ °C)/\ \theta s(55\ °C) = 105/42 = 2.5$

$Q1 = 2.50.1 = 1.096$

$\Delta T = 45\ °C – 35\ °C = 10\ °C$

$Q\Delta T = Qs(35\ °C)/Qs(45\ °C)$

$1.09610 = Qs(35\ °C)/105$

$2.5 \times 105 = Qs(35\ °C)$

$Qs(35\ °C) = 262.5$ days

The IMPD was produced from IPM. IPM was safe for consumption because the oil extraction process of IPM was conducted using hexane and the hexane residue in IPM was rechecked using the IUPAC method 2.607 (1987). If ingested food contains hexane, it may cause severe abdominal pain and impact the respiratory system, resulting in shortness of breath, coughing, burning of the mouth, throat or chest and even chemical pneumonitis. Moreover, IPM had already been cooked by boiling and dried in a hot air oven before being used as a main ingredient in the IMPD. The protein hydrolysis process also used food grade Bromelain enzyme and was also checked for pathogenic microorganisms before ingestion. This microbiological quality until the end of shelf life could confirm that this IMPH powder was safe to consume. One pack (50 g) of IMPD with cocoa flavor blended with hot water (250 mL) provided 160.72 kcal, and was high in protein and dietary fiber. Therefore, this product is suitable for people who have difficulty digesting Inca peanut protein and for people with digestive system problems such as patients with cancer, the elderly and patients with weakened muscles.

Table 14 Sensory acceptance scores of IMPD with cocoa flavor during storage at 35, 45 and 55 °C for 0 to 12 weeks.

Temperature (°C)	Storage time (week)	Appearance	Color	Aroma	Taste	Viscosity^{ns}	Overall acceptability
35	0	8.90 ± 0.65^{a}	8.32 ± 0.55^{a}	8.06 ± 0.47^{a}	7.94 ± 0.37^{a}	8.06 ± 0.79	8.65 ± 0.64^{a}
	3	8.57 ± 1.23^{a}	8.01 ± 0.96^{ab}	8.12 ± 1.74^{a}	7.68 ± 0.87^{a}	7.96 ± 0.79	8.72 ± 0.87^{a}
	6	8.48 ± 0.51^{a}	8.32 ± 0.55^{a}	8.06 ± 0.47^{a}	7.88 ± 0.37^{a}	8.11 ± 0.79	8.58 ± 0.44^{a}
	9	8.17 ± 0.12^{ab}	7.91 ± 0.81^{b}	7.27 ± 1.24^{b}	7.43 ± 0.94^{ab}	7.83 ± 1.12	7.95 ± 0.11^{b}
	12	8.19 ± 0.04^{ab}	6.45 ± 0.01^{c}	6.72 ± 1.11^{c}	7.10 ± 1.34^{b}	7.77 ± 0.72	7.70 ± 1.22^{b}
45	0	8.90 ± 0.65^{a}	8.32 ± 0.55^{a}	8.06 ± 0.47^{a}	7.94 ± 0.37^{a}	8.06 ± 0.79	8.26 ± 0.44^{a}
	3	8.16 ± 1.54^{ab}	7.82 ± 1.05^{b}	7.16 ± 1.31^{b}	6.94 ± 1.14^{b}	8.23 ± 0.91	7.92 ± 0.62^{b}
	6	7.85 ± 0.04^{b}	7.73 ± 1.34^{b}	7.03 ± 1.27^{b}	6.54 ± 0.14^{bc}	8.00 ± 0.20	6.94 ± 1.25^{c}
	9	7.72 ± 0.15^{b}	7.74 ± 0.03^{b}	6.76 ± 1.27^{c}	5.75 ± 1.95^{c}	7.73 ± 0.23	6.43 ± 1.03^{c}
	12	7.25 ± 0.03^{bc}	7.46 ± 0.02^{b}	6.52 ± 1.00^{c}	5.23 ± 0.03^{c}	7.89 ± 0.12	5.58 ± 1.22^{d}
55	0	8.90 ± 0.65^{a}	8.32 ± 0.55^{a}	8.06 ± 0.47^{a}	7.94 ± 0.37^{a}	8.06 ± 0.79	8.26 ± 0.44^{a}
	3	8.29 ± 1.59^{ab}	8.11 ± 0.82^{a}	5.69 ± 1.78^{b}	5.94 ± 0.37^{b}	8.24 ± 0.96	5.26 ± 0.44^{b}
	6	6.36 ± 0.02^{c}	5.84 ± 0.05^{b}	4.64 ± 1.74^{c}	4.85 ± 0.26^{c}	8.07 ± 1.11	4.29 ± 0.61^{c}
	9	-	-	-	-	-	-
	12	-	-	-	-	-	-

These results were expressed as mean ± standard deviation (SD) from triplicate determination. $^{a-e}$ Means followed by different letters within the same column indicate significant differences ($p \leq 0.05$). ^{ns} Not significant ($p > 0.05$).

Conclusions

The study demonstrates that optimizing the maltodextrin content and inlet air temperature significantly impacts the yield, protein content, and other physicochemical properties of Inca peanut meal protein hydrolysate (IMPH) powder. Increasing maltodextrin content consistently enhances yield while reducing protein content across all tested temperatures (150, 160 and 170 °C). For instance, at 170 °C, the yield increased from 19.24 to 45.63 % as maltodextrin levels rose from 5 to 15 %, whereas protein content dropped from 34.35 to 28.28 %. The optimal condition for spray drying the IMPH, involving the addition of 10 % maltodextrin and an inlet temperature of 160 °C, provided the best overall results, including high yield, protein content, water solubility index (WSI) and lower moisture content and water activity. IMPH powder produced under these optimal conditions showed the highest total phenolic content (TPC), antioxidant activities, and the highest sensory scores for appearance, color, aroma, taste, viscosity and overall acceptability. The IMPD with cocoa flavor, derived from IMPH, exhibited the highest TPC values, antioxidant activities, and sensory scores, indicating its potential as a high-protein and high-dietary fiber product. The cocoa-flavored IMPD could be stored in laminated zip lock plastic bags (size 12×18 cm^2) at 35 °C for 262.5 days while retaining acceptable sensory properties. Moreover, the total plate count, yeast and mold count of this product were within the limits set by the Thai Industrial Standards Institute (TISI 688/2547). These findings suggest that optimizing maltodextrin levels and inlet temperature can enhance

production efficiency while maintaining the nutritional quality of the final product, thereby meeting market demands effectively.

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