Abstract

Objectives

The edible bird’s nest hydrolysates (EBNH) are

Introduction

All sectors of the food product development venture are still addressing the changing needs to meet the present demands of consumers for newer alternatives novel healthy foods (Guiné *et al*., 2020), which shows the rising popularity of instant foods such as beverages in powder form (Ren *et al*., 2024). Powder drink mix (PDM) is a food category that is becoming more popular and is considered capable of sustaining its position as an important food since it offers the ideal combination of convenience and functional benefits. Consumers can enjoy a variety of powdered drinks in the market that are flavourful, nutritious, safe, convenient, colourful and affordable (Çopur *et al*., 2019). One of the major concerted research efforts across the globe is the development of plant-based or non-dairy milk alternatives of newer or specialty beverage products with functionality. Some PDM is formulated to address problems such as cow milk allergy related to lactose intolerance, calorie concerns, and the prevalence of hypercholesterolemia (Sethi *et al*., 2016).

Carbohydrates from foods are commonly consumed macronutrients that serve as the primary energy source

enhancing foods that are low in calories for effective weight management have been developed and can be consumed as a meal to satisfy hunger (Smethers and Rolls, 2018; Kim, 2021). Reducing calorie intake should not be accompanied by a disproportionate reduction in nutrient needs. Consuming a nutrient-rich diet to protect nutritional status and health is essential for anyone trying to lose weight (Blum, 2021).

Despite the surge in the popularity of plant-based powder drink mixes, only a few inspiring formulations with antioxidant potential have been reported. Such food products were formulated using sorghum (Queiroz *et al*., 2018), vegetables (Bochnak-Niedźwiecka *et al*., 2020), mango (Akther *et al*., 2020) and raw banana (Budnimath *et al*., 2023), but without enrichment with animal protein hydrolysates (Etemadian *et al*., 2021). Malaysia is a country engaged in swiftlet industry (Yaa'cob *et al*., 2021) that produces edible's bird nests (EBN) as a safe and nutritious source of animal glycoprotein hydrolysates for human consumption (Ghassem *et al*., 2017; Din *et al*., 2022). Edible bird nest is recognized to offer several numerous therapeutic benefits, including anti-viral, anti-cancer, neuroprotective effects, cognitive

novel medications with antioxidant and antihypertensive effects, such as celiprolol, is being developed to be taken as antioxidant medications to improve antihypertensive treatment (Nawarskas *et al*., 2017; Amponsah-Offeh, 2023). A powder drink mix containing EBNH could provide an attractive therapeutic beverage to avert hypertension and inflammation.

We have previously detailed the enzymatic hydrolysis process of EBN from swiftlet (*Aerodramus fuciphagus*) using alcalase enzyme to produce spray-dried EBNH (Mohd Aris *et al*. 2023). We also successfully created a cocoa-flavoured soy-based carbohydrate PDM that contained EBNH. Due to its high solubility, the EBNH was introduced as an ingredient at the rate of 0.15 g per 5 g or 3.0% (w/w). The other ingredients of the PDM are 75.44% (w/w) soybean powder, 20% (w/w) cocoa powder, 0.5% (w/w) xanthan gum, 1.0% (w/w) silicon dioxide and 0.06% (w/w) sucralose. Statistical response surface methodology, sensory evaluation and product proximate analysis were used in an integrated approach to establish the formulation of the PDM.

The quality of PDM as a functional food is enhanced by the addition of EBNH as a component. By transforming EBNH into a more palatable consumable form, the PDM may encourage more people to take EBN bioactive glycopeptides for health benefits. The PDM is a novel food product that provides an alternative to ingesting encapsulated EBNH or directly consuming EBN, which is often double-boiled with rock sugar and turned into soup (Lee *et al*., 2021). Babji *et al*. (2015) encapsulated EBNH which can be taken daily as a supplement with bioactive compound (each weighing 0.15 g or 0.62 cm^3). The formulated PDM is an enticing product that is not only nutritious, tasty, sugar-free, lactose-free, has a neutral pH, is sustainable, and has shown to have antioxidant activity. Our previous research demonstrated that the addition of EBNH to PDM significantly increased the product's overall in vitro antioxidant capacity (Mohd Aris *et al*., 2023). The ABTS and Folin-Ciocalteu reag0052006(dem)15(onst)-6(r)-3(a)9(2.024 330.7<0057>-4<024 710.0

Materials and Methods

Product physicochemical properties analysis

The PDM samples were prepared in accordance with its established formulation containing EBNH that was acquired using the enzymatic hydrolysis procedure of EBN that we had described in our previous study (Mohd Aris *et al*., 2023). Using the AOAC (2006) methodologies, the proximate analysis of PDM and its ingredients were conducted with respect to their constituents of water, ash, crude protein, crude fat and dietary fibre. The values of the constituents were used to calculate the percentage of available carbohydrate according to the formula: % available carbohydrate = 100 - moisture (%) - ash (%) - crude protein (%) - crude fat (%) - dietary fibre (%). The contents of crude protein, crude fat, and carbohydrate are multiplied by the specific calorie equivalent factors to get the estimated energy value of the formulated product (Food

Angiotensin-converting enzyme (ACE) inhibitory activity

Using the simple and sensitive method proposed by Li *et al*. (2005), the in vitro ACE inhibitory activity was ascertained using the water extracts of PDM and all of its ingredients. The method involves measuring the spectrophotometric absorbance of the released hippuric acid (HA) at 492 nm. Hippuryl-l-histidyl-l-leucine (HHL), ACE of rabbit lung (≥ 2.0 unit/mg protein), sodium borate, sodium chloride, hydrochloric acid, quinoline, benzene sulfonyl chloride (BSC) and captopril were purchased from Sigma-Aldrich (St. Louis, USA).

The solutions of PDM (fortified and non-fortified with EBNH) and the ingredients (soybean powder, cocoa powder, EBNH, silicon dioxide, xanthan gum, and sucralose) were prepared at concentrations of 1 mg/mL, 0.97 mg/mL, 0.7544 mg/mL, 0.2 mg/mL, 0.03 mg/mL, 0.01 mg/mL, 0.005 mg/mL and 0.0006 mg/mL, respectively. For each respective solution, a volume of 20 µL was added to the assay mixture comprising 50 µL of 5 mmol/L HHL in 100 mmol/L pH 8.3 sodium borate buffer (containing 300 mmol/L NaCl), pre-incubated at 37 ºC for 5 minutes before adding 10 µL of ACE solution (100 mU/mL). The mixture was then incubated for an additional 30 minutes at 37 ºC. The reaction was stopped by adding 100 µL of 1M HCl. Then, 320 µL of

The membrane stabilization activity was estimated by measuring the degree of hemolysis of RBC in a hypotonic environment (Anosike *et al*., 2012; Kumari *et al*

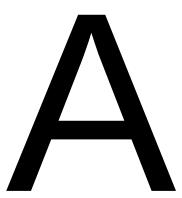
consisting of 51 items for self-assessment to aid in determining their three cognitive and behavioural domains of eating: cognitive restraint, disinhibition and hunger (Stunkard and Messick, 1985). Thirty eligible subjects were then selected based on an acceptable BMI of 18.5-24.9 (National Institute of Health, 2024) and TFEQ scores. A subject is considered to have normal eating habits if he or she has a score of ≤10 for the category of restraint and disinhibition, and a score of ≤7 for hunger (Stunkard and Messick, 1985). The selected subjects were instructed to fast overnight for 10-12 hours and consume only water prior to the morning of the testing sessions. The subjects' 2

$$\text{Glycemic index (GI)} = \frac{\text{2-hour iAUC postprandial blood glucose of the PDM meal}}{\text{2-}} \times 100$$

6 g per 100 g weight (Stephen *et al*., 2017). A 250 mL serving of PDM meal provides 12.8 g of fibre, while consuming 240 mL of the product as a nutritious beverage provides 4.9 g of fibre. A serving of a food item is considered a ‘good source of fibre’ and ‘high in fibre’ when it contains 2.5 to 4.9 g and ≥5.0 g of fibre, respectively (Li and Komarek, 2017).

Table 1. Proximate composition and energy of PDM with its ingredients in 5 g weight sample

Ingredients/PDM	Moisture (%)	Ash (%)	Protein (%)	Fat (%)	Carbohydrate (%)	Energy (kcal/100g)
Soybean powder	5.53 ± 0.1^{A}					



The product, whether in the form of a beverage or a satiating meal, exhibits a shear thinning nature with distinct characteristics of pseudo-plastic behaviour. It falls under the classification of a non-Newtonian fluid (n<1), as discussed by Björn *et al*. (2012). The beverage and satiating meal of PDM have different textures), with consistency indices of 0.39 ± 0.09 $Pa.s^n$ as shown in Figure 2a and 14.17 ± 1.74 $Pa.s^n$ as shown in Figure 2b, respectively. Their flow behaviour indices are 0.42 ± 0.04 and 0.39 ± 0.002, respectively. The pH of the beverage is almost neutral (6.9 ± 0.04), so drinking PDM as a beverage is not expected to decrease the pH of the mouth to be less than 5.5, which could pose a threat to dental erosion (Rusu *et al*., 2022).

Product functional effects

The hot water extract (90 - 95 ^oC) of the PDM (a beverage with a concentration of 12.5% (w/v) and a consistency index of 0.39 ± 0.09 $Pa.s^n$) was observed to be capable of indicating the reactivity of EBNH in relation to its in vitro functional effects concerning ACE inhibitory activity and erythrocyte membrane stabilization activity.

affinity for the C- and N- domains of ACE (Yates *et al*., 2014), since the enzymatic activity of ACE depends on the presence of chloride ions which is unique among the metallopeptidase family (Fang *et al*., 2019).

Blood pressure tend to increase with ageing. Men are more likely than women to have high blood pressure among those under the age of 65 (Everett and Zajacova, 2015). Although rise in blood pressure typically affects adults, children and teenagers may also experience high blood pressure (Azegami *et al*., 2021). Research indicates the age for early-onset hypertension is at ≤ 55 years old (Suvila *et al*., 2020). Young adults suffering from hypertension is common nowadays, affecting 1 in 8 youngsters aged between 20 and 40 years old (Hinston *et al*., 2019). Unhealthy diet, obesity, unhealthy lifestyle habits, and race or ethnicity are some possible risk factors linked to early-onset hypertension (Huang *et al*., 2022) High blood pressure in young adults (which may be brought on by stress and strenuous exercise) may be linked to a high level of renin enzyme in blood that is released by the kidney (Ayada *et al*., 2015; Deja *et al*., 2022). People younger than 55 years tend to benefit more from ACE inhibitors (Izzo and Weir, 2011; National Institute for Health and Care Excellence, 2019). Renin acts on angiotensin to form angiotensin I, which is finally converted by the action of ACE to form angiotensin II which causes hypertension (Ghazi and Drawz, 2017). Since PDM has the capability to inhibit ACE, regular and consistent consumption of such beverage may substantially block the renin-angiotensin-aldosterone pathway, preventing ACE from converting angiotensin to angiotensin II (Arendse *et al*., 2019). Apart from achieving better blood pressure control, this novel therapeutic approach has the potential to provide improved cardiovascular protection without adverse side effects.

Erythrocyte membrane stabilization activity

The method in the study employed spectrophotometric measurement to determine the amount of hemoglobin released from RBC due to in vitro induced hemolysis using hypotonic stress. This methodology has its limitation due to the interference in the hemolysis processes. The presence of several constituents by intracellular release from RBC that may generate negative impact effects on the chemical reactivity of analytes from the test samples, may cause bias estimation on the real values (Unlu *et al*., 2018). In this process, not only the hemoglobin is released but also other components of the erythrocyte cytoplasm. Nevertheless, in vitro-induced hemolysis can still be a reliable diagnostic approach if blood sample collection and handling are done appropriately to eis

We expected all treatments in the study to cause hemolysis of RBC; however, the results showed that this did not happen with water extracts of PDM's food additives (silicon dioxide, xanthan gum, and sucralose). The water extract of soybean powder (5 g in 40 mL of hot water at 90 - 95 ^{o}C) exhibited the

placed in a hypotonic condition. This could lead to RBC enlarging and hemolyzing because of a weakening cell membrane. In the case of

(Yedjou *et al*., 2023). The PDM is a sugar-free product containing antioxidants from EBNH and plant-based ingredients, can potentially prevent cell damage and combat inflammation in humans.

Product glycemic response

Table 2 shows the characteristics of 12 healthy subjects (9 females, 3 males) who participated in the PDM trial on glycemic response. Their average age, weight, height, and BMI were 26.9 ± 1.9 years, 57.6 ± 6.4 kg, 1.59 ± 0.06 m, and 22.6 ± 1.3 kgm^{-2}, respectively. For TFEQ, the subjects' average scores for food restraint, disinhibition, and hunger were 6.6 ± 2.8, 4.9 ± 2.1, and 4.5 ± 2.5, respectively, indicating they had normal eating habits. The average fasting blood glucose values (baseline glucose level) of the subjects were considered stable since the difference between two measurements at different test times (5.2 mmol/L versus 5.3 mmol/L) was not significant (p>0.05). A non-diabetic person is considered normal to have a blood glucose level of 3.9 - 5.5 mmol/L when fasting (Mathew *et al*., 2023).

Table 2. Subjects' characteristics involved in trials of glycemic and satiety impact studies

Measurements	**Glycemic response**			**Satiety response**		
	Female (n=9)	**Male (n=3)**	**Total (n=12)**	**Female (n=20)**	**Male (n=10)**	**Total (n=30)**
Age (years)	26.8 ± 2.0	27.3 ± 2.1	26.9 ± 1.9	29.2 ± 4.6	27.2 ± 3.2	28.5 ± 4.2
Weight (kg)	55.4 ± 4.9	64.3 ± 6.5	57.6 ± 6.4	56.8 ± 5.8	66.4 ± 6.3	60.0 ± 7.5
Height (m)	1.57 ± 0.05	1.65 ± 0.04	1.59 ± 0.06	1.59 ± 0.05	1.67 ± 0.07	1.62 ± 0.07
BMI (kgm^{-2})	22.3 ± 1.0	23.5 ± 1.9	22.6 ± 1.3	22.4 ± 1.8	23.8 ± 1.1	22.8 ± 1.7
TFEQ (*restraint*)	7.0 ± 3.0	5.3 ± 2.3	6.6 ± 2.8	7.0 ± 2.4	7.4 ± 2.2	7.1 ± 2.3
TFEQ (*disinhibition*)	4.6 ± 2.1	6.0 ± 1.7	4.9 ± 2.1	7.0 ± 2.4	5.1 ± 1.4	5.1 ± 1.9
TFEQ (*hunger*)	4.2 ± 2.4	5.3 ± 2.9	4.5 ± 2.5	4.6 ± 1.9	5.9 ± 1.7	5.0 ± 1.9

Note: BMI = Body Mass Index; TFEQ = Three Factor Eating Questionnaire

Figure 6 shows the changes in the average blood glucose level of the 12 subjects over time for a period of 2 hours after ingesting 250 mL of each meal of PDM and glucose standard solution. The blood glucose average values of PDM (mean 5.6 ± 0.2 mmol/L) was significantly lower (p<0.05) than that of

due to its ingested quantity of carbohydrates (Kim, 2020). The GL value is categorized as low since it is less than 10 (Ramdath, 2016; Venn and Green, 2007). On the other hand, assuming a person drinks PDM as a beverage (12.5% w/v) containing 5.0 g per 40 ml 90-95^{o}C hot water), which is equivalent to 240 mL

Table 3. Subjects' perceptions on the quality of the glucose solution and the PDM meal.

Perceptions	Food samples	Female (n=20)	Male (n=10)	All (n=30)
How many times did you have to chew the test sample before you actr602.6172.0eD 5/				

the difference between the AUC values of glucose and PDM (687.09 versus 6320.75) was also significant ($p<0.05$), implying that the PDM meal was nine times more satiating than the glucose solution. The correlation between the satiety scores and the 2-hour rise of postprandial blood glucose (<6 mmol/L) was not significant ($R^2 = 0.31$, $p>0.05$). Overall, the PDM meal suppressed hunger for approximately 2.5 hours.

subject, as well as the environment in which the food and the subject interact (Johnstone and Stephen, 2020).

A food's energy density (calories per gram) depends on the mixture of its macronutrients, fibre, and water. The incorporation of water decreases ED but increases volume and weight (Lovegrove *et al*., 2017) of PDM. The ED of foods ranges from 0 to 9 calories per gram (Rolls, 2017), or can be categorized as very-low ED (<0.6 kcal/g), low ED (0.6 - 1.5 kcal/g), medium ED (1.6 - 3.9 kcal/g), and high ED (4.0 - 9.0 kcal/g). The PDM meal that was served in the trial is a medium energy-

2.5 hours. Drinking PDM on a regular basis may also assist consumers in reducing their intake of excess calories, which is good news for

Author Contributions

Hazimah Mohd Aris: Formal Analysis (Lead), Investigation (Equal), Methodology (Lead), Writing Original Draft (Lead); *Zalifah Mohd Kasim:* Formal Analysis (Equal), Investigation (Lead), Methodology (Lead), Supervision (Lead), Data Validation (Equal), Writing, Review and Editing (Lead); and *Saiful Irwan Zubairi:* Supervision (Supporting), Data Validation (Equal), Review and Editing (Verification).

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Figure Captions

Figure 1. Schematic line scale of the labelled bipolar magnitude satiety rating scores

Figure 2. Pictures of the PDM beverages prepared in 90 - 95 ^{o}C

Figure 1

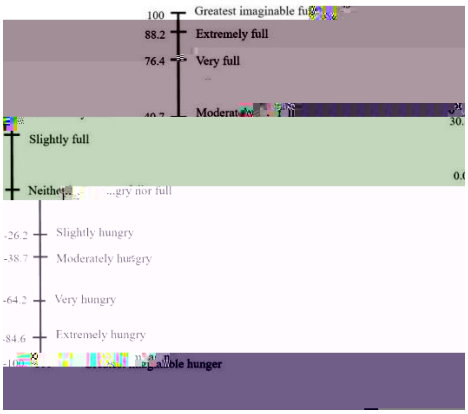

Figure 2

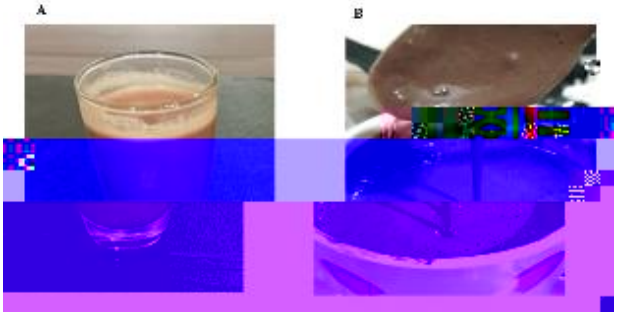

Figure 3

Figure 4

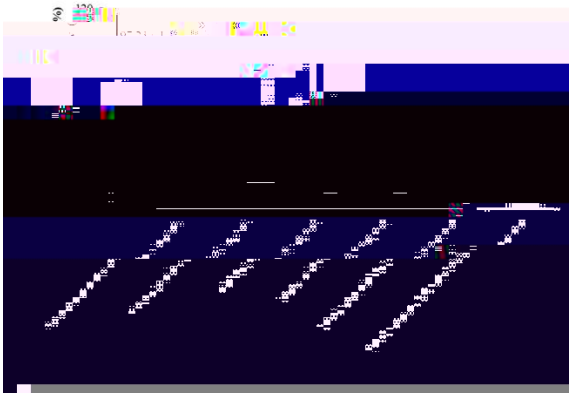

Figure 5

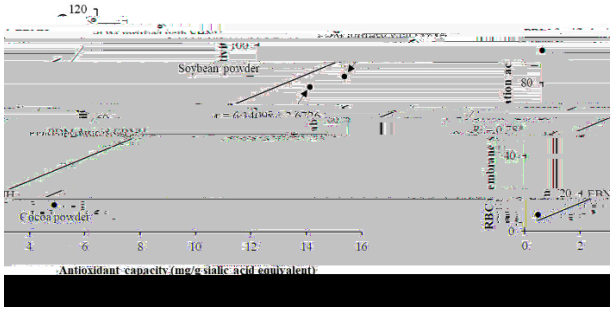

Figure 6

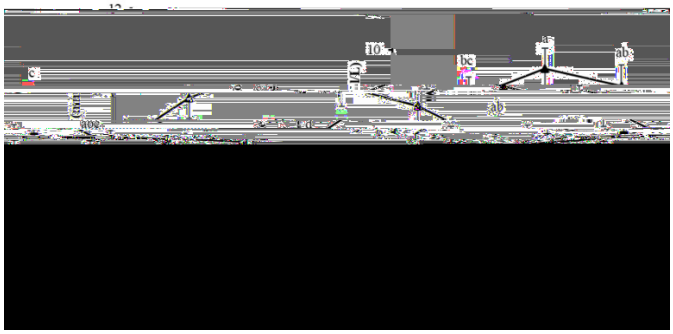

Figure 7