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Research Article

Food Insecurity and Cardiometabolic Risk among Turkish Adults: A Cross-Sectional Study

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ABSTRACT

This study examined 175 volunteers aged 18–64 to explore the connection between food insecurity and anthropometric measurements, diet quality scores and cardiometabolic risk factors. The design of the study is cross-sectional, and the data were obtained using a number of questionnaires applied to individuals who visited the diet clinic. Participants' diet quality was assessed using the Healthy Eating Index-2015 (HEI-2015) and food security was evaluated using the Household Food Insecurity Access Scale. Cardiometabolic risk factors were determined based on HEI-2015 scores, Body Mass Index (BMI), waist circumference, waist/height ratio, physician-diagnosed diabetes, hypertension, dyslipidemia, regular physical activity status, and smoking habits. As a result of the study, 41.1% of the participants were found to be food-insecure, and 77.8% had diet quality that needed improvement. The average BMI value of food-insecure women was found to be higher than that of food-secure women (32.37±7.77 kg/m^2 and 29.86±5.22 kg/m^2, respectively) (p=0.003). Furthermore, food-insecure women had a higher average waist circumference (p=0.001). A significant negative relationship was determined between BMI value, waist circumference and waist/height ratio, which are among the cardiometabolic risk factors, and food insecurity.

INTRODUCTION

Food security involves the continuous availability of clean, safe, and nutritious foods, as outlined by the FAO, which emphasizes availability, access, utilization, and sustainability (FAO 1996). Conversely, food insecurity, the inability to obtain necessary nutrients and sufficient food, affects billions globally (FAO 2022). This issue is exacerbated in developing countries due to population growth, low production, and weak markets, leading low-income families to consume cheaper, less nutritious options (Maiangwa *et al.* 2010). Such diets, high in unhealthy fats and low in nutritional quality, contribute to cardiometabolic diseases like diabetes, obesity, and metabolic syndrome (Laraia 2013).

Cardiometabolic diseases are a global health concern (Kılıçkap *et* al. 2018), closely linked to nutrition. Poor diets and food insecurity increase risks, particularly in developing countries (Kontas *et al.* 2014). Unhealthy diets cause plaque buildup and inflammation, raising cardiometabolic risks (Cardoso Lde *et al*. 2016). Balanced diets with appropriate fats are essential, but food insecurity limits access to quality foods (Eicher-Miller & Zhao 2018).

Food insecurity also correlates with obesity, as low-income individuals resort to inexpensive, high-fat foods, increasing body fat (Nettle *et al.* 2017). Stress-induced emotional eating worsens obesity (Rasmusson *et al.* 2018), while poor diets impair glycemic control (Beltrán *et al.* 2022) and heighten Hypertension (HT) risk due to inadequate nutrient intake (Helmick *et al.* 2018). Smoking further amplifies cardiometabolic risks (Levine *et al.* 2013; Pan *et al*. 2015; Hackshaw *et al*. 2018).

This study aims to assess food insecurity among participants and explore its relationship with cardiometabolic risk.

METHODS

Design, location, and time

This cross-sectional study took place in Gölcük District Health Directorate, Kocaeli Province, Türkiye, from October to December 2020. The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Institutional Review Board (or Ethics Committee) of the Institute of Health Sciences of the University of Health Sciences in Istanbul, Türkiye (protocol code 20/352 and date of approval 25 September 2020).

Sampling

A total of 175 volunteers aged 18–64 participated, excluding pregnant and lactating women. The number of individuals visiting the diet clinic of the community health center where the study was to be conducted was 244. Accordingly, it had been calculated that the sample size for the study should be a minimum of 150 individuals with a 95% confidence interval and a 5% precision (Samp Size Calculator 2023).

Data collection

Face-to-face interviews were conducted, gathering demographic, health and dietary information using a questionnaire. Additionally, Retrospective 24-Hour Food Consumption Records and the Household Food Insecurity Access scale were utilized. Educational, marital and employment statuses were recorded through a 23-question survey. Anthropometric measurements and body composition analysis (TANITA BC418 MA) were performed.

Food insecurity assessment-2007. The Household Food Insecurity Access Scale (2007) was developed by the United States Agency for International Development to determine the levels of food insecurity of individuals. The score from the scale is a continuous measure of the degree of food insecurity in a household over the past four weeks (30 days). There are nine questions with a four-point Likert structure combining each occurrence and frequency question out of a total of 18 questions. If a participant experienced the situation stated in an occurrence question, an accompanying frequency question would explore how often he or she experienced such a situation during the last four weeks (0=never, 1=rarely, 2=sometimes, 3=often). If the participant's answer to the occurrence question was “no”, he or she was asked to move on to the next occurrence question without answering the frequency question for that occurrence question. The food insecurity score was calculated by summing up the scores of the frequency questions for the occurrence questions for which “yes” was the answer. As it was a categorical variable, households were categorized either as food-secure or as food-insecure. The answers to the questions (never=0 points, rarely=1 point, often=3 points) were scored. All answer scores were collected. It has been stated that the severity of food insecurity increases as the total score on the scale increases. Specifically, households with a total score of 0-1 are clasiffied as food-secure, while households with a score of 2 or higher are classified as food-insecure (Coates *et al.* 2007).

Diet quality evaluation**.** Through an analysis of food consumption records, the average daily intake of energy and nutrients for each individual was calculated. The Nutrition Information System (BeBIS 15.0) computer software was employed to assess the energy and nutrient contents derived from the participants' daily dietary intake. The energy and nutrient values were used to calculate the HEI-2015 scores of individuals. HEI-2015 was used to evaluate individuals' diet quality. It consists of 13 components. Nine components—total fruits, whole fruits, total vegetables, dark green leafy vegetables and legumes, whole grains, milk and dairy products, total protein foods, seafood and vegetable proteins and fatty acids—constitute the components of the index that should be taken in sufficient quantities. Refined foods, sodium, added sugar and saturated fat are the components of the index that should be reduced. Individuals' 24-hour food consumption was recorded, and their average daily calorie intake was calculated. In the index, a reference score corresponding to 1,000 kcal is given for each food group. According to his or her calorie intake, an individual's score for that food group was calculated using ratio and proportion. The total score was obtained by adding up the scores from all food groups. The highest score of the index is 100; scores ≤50 indicate “poor diet quality”, scores of 51–80 indicate a “need to be improved” and scores ≥80 indicate “good diet quality” (Krebs-Smith *et al.* 2018).

BMI, waist circumference and waist/height ratio. BMI value and waist circumference were measured to evaluate cardiometabolic risk. BMI value was calculated by dividing weight (kg) by the square of height (m^2). BMI was classified according to World Health Organization (WHO 1995) criteria. Waist circumference, indicative of abdominal fat, was categorized based on gender-specific thresholds. For men, a waist circumference <94 cm is indicative of low risk for chronic diseases, a waist circumference between 94 and 102 cm is indicative of high risk and a waist circumference >102 cm is indicative of very high risk. For women, a waist circumference <80 cm is indicative of low risk, a waist circumference between 80 and 88 cm is indicative of high risk and a waist circumference >88 cm is indicative very high risk (WHO 1995). Waist/height ratio, a recent criterion for obesity and cardiometabolic risk, was calculated using waist circumference and height. A ratio below 0.5 indicates low risk (WHO 2000). Hip circumference was measured at a level parallel to the floor at the largest circumference of the buttocks. Other data of anthropometric measurements used in the study—body weight, body fat mass and percentage, lean body mass and percentage and body water content ratio—were obtained and recorded by the researcher with TANITA BC418 MA.

Cardiometabolic diseases and physical activity. Diagnoses of diabetes, hypertension and dyslipidemia were made by the physician taking into account certain criteria. An oral glucose tolerance test was performed when the fasting blood glucose was at the level of 126 mg/dL or higher. Diabetes was diagnosed after the individual drank 200 mL of sugar water and the blood glucose value rose beyond 200 mg/dL after the 2nd hour (Nkonge *et al.* 2020). Hypertension was diagnosed by measuring the blood pressure. The diagnosis was made when the systolic blood pressure was constantly at 140 mmHg or above and the diastolic blood pressure was at 90 mmHg (Jones *et al.* 2020). Plasma total cholesterol ≥190 mg/dL, LDL cholesterol ≥130 mg/dL and HDL cholesterol ≤50 mg/dL in women (≤40 mg/dL in men) led to a dyslipidemia diagnosis (Berberich & Hegele 2022). Diagnoses were made by specialist physicians. To evaluate their physical activity level, individuals were asked whether they did any regular physical activity.

Data analysis

The NCSS (Number Cruncher Statistical System) 2007 (Kaysville, Utah, USA) program was used for the statistical analysis of the data obtained from the research. A chi-square analysis was used to determine the relationship between qualitative data. Simple and multiple logistic regression analyses were used to determine the independent variables affecting the dependent variable. The dependent variable was determined as food security status, and the independent variables were determined as age, BMI value, waist circumference, waist/height ratio, education level (primary school and university levels) and employment status (housewife included). The statistical significance in the analyses was evaluated at $p<0.05$.

RESULTS AND DISCUSSION

Information on the demographic characteristics such as education status and socioeconomic status of the food-secure and food-insecure participants is presented in percentages (%) in Table 1. The percentages of male and female representation were 11.4% and 88.6%, respectively. It was determined that 41.1% of the participants in the study were food-insecure, 64% were aged 35–64 and 76.6% were married. There was a significant difference in education levels between individuals with food security and those without food security (p=0.001). The majority of the individuals, both with and without food security, were housewives (36.9% and 66.7%, respectively). A significant difference was observed in working statuses between food-secure and food-insecure individuals (p=0.001). The average monthly total income of most households with food security (47.5%) was 5,000 Turkish lira ($676). While most of the food-secure individuals (51.5%) stated that their income was equal to their expenses (for nutrition, clothing, shelter, etc.), most of the food-insecure individuals (52.8%) reported that their income was less than their expenses.

In Table 2, the number and percentage (%) values of diet quality of the food-secure and food-insecure participants according to their cardiometabolic characteristics are presented. Most of the participants (n=127) had diet quality that needed improvement. The majority of the participants were obese. Anthropometroic measurements by food security status for males and females are detailed in Table 3.

Table 4 shows the results of a simple logistic regression analysis performed to determine the effect of age, BMI value, waist

Table 1. Participants' descriptive characteristics according to their food security status

Variables	Total (n=175)	Food insecure (n=72)	Food secure (n=103)	*p*
	n (%)	n (%)	n (%)	
Gender				
Female	155 (88.6)	64 (88.9)	91 (88.3)	0.556
Male	20 (11.4)	8 (11.1)	12 (11.7)	
Age (years)				
18–34	63 (36.0)	21 (29.2)	42 (40.8)	0.078
35–64	112 (64.0)	51 (70.8)	61 (59.2)	
Education status				
<High school	67 (38.3)	45 (62.5)	22 (21.4)	<0.001[**]
≥High school	108 (61.7)	27 (37.5)	81 (78.6)	
Marital status				
Single	41 (23.4)	17 (23.6)	24 (23.3)	0.962
Married	134 (76.6)	55 (76.4)	79 (76.7)	
Working				
Officer	26 (14.9)	1 (1.4)[a]	25 (24.3)[b]	0.001[**]
Worker	14 (8.0)	3 (4.2)	11 (10.7)	
Retired	6 (3.4)	2 (2.8)	4 (3.9)	
Self-employment	14 (8.0)	6 (8.3)	8 (7.8)	
Housewife	86 (49.1)	48 (66.7)[a]	38 (36.9)[b]	
Other	29 (16.6)	12 (16.7)	17 (16.5)	
Social insurance				
Yes	155 (88.6)	60 (83.3)	95 (92.2)	0.058
No	20 (11.4)	12 (16.7)	8 (7.8)	
Number of household members				
≤2	32 (18.3)	12 (16.7)	20 (19.4)	0.893
3–5	121 (69.1)	51 (70.8)	70 (68.0)	
>5	22 (12.6)	9 (12.5)	13 (12.6)	
Income of family (TL)				
<3,000	61 (34.8)	43 (59.7)	18 (17.5)	<0.001[**]
3,000–5,000	57 (32.6)	21 (29.2)	36 (34.9)	
>5,000	57 (32.6)	8 (11.1)	49 (47.5)	
Economic situation				
Income>Expense[***]	28 (16.0)	2 (2.8)	26 (25.2)	<0.001[**]
Income=Expense[***]	85 (48.6)	32 (44.4)	53 (51.5)	
Income<Expense[***]	62 (35.4)	38 (52.8)	24 (23.3)	

TL: Turkish Lira (1$=~7.5TL);[*]A chi-square test p<0.05, [**]p<0.01; [***]: Nutrition, clothing, shelter, bills, transportation, education, personal expenses, etc. are included in the expenses

circumference, waist/height ratio, educational status (primary school and university level) and employed or housewife status on food insecurity. There was a significant negative relationship between age and food insecurity. As age increased, the frequency of food insecurity in individuals decreased. A negative relationship was also observed between individuals' BMI values and food insecurity ($p<0.001$). Table 4 further shows that waist circumference had a significant negative relationship with the food insecurity score. Furthermore, the regression coefficient showed that the waist/height ratio ($\beta=0.003$, $p<0.001$) had a significant negative

Table 2. Participants' cardiometabolic characteristics according to their food security status

Cardiometabolic characteristics	Total (n=175)	Food insecure (n=72)	Food secure (n=103)	*p*
	n (%)	n (%)	n (%)	
Healthy eating index score				
Poor diet quality	37 (21.1)	11 (15.3)	26 (25.3)	0.282
Need to be improved	127 (72.6)	56 (77.8)	71 (68.9)	
Good diet quality	11 (6.3)	5 (6.9)	6 (5.8)	
Body mass index (kg/m^2)				
Underweight (<18.50)	3 (1.7)	3 (4.2)	0 (0.0)	0.001**
Normal (18.50–24.99)	23 (13.1)	6 (8.3)	17 (16.5)	
Overweight (25.00–29.99)	64 (36.6)	18 (25.0)	46 (44.7)	
Obese (≥30.00)	85 (48.6)	45 (62.5)	40 (38.8)	
Waist circumference (cm)				
Low risk	30 (17.1)	9 (12.5)	21 (20.4)	0.042*
High risk	53 (30.3)	17 (23.6)	36 (34.9)	
Very high risk	92 (52.6)	46 (63.9)	46 (44.7)	
Waist/Height ratio				
Low risk	26 (14.9)	7 (9.7)	19 (18.4)	0.082
High risk	149 (85.1)	65 (90.3)	84 (81.6)	
Diagnosis of diabetes				
Yes	26 (14.9)	11 (15.3)	15 (14.6)	0.530
No	149 (85.1)	61 (84.7)	88 (85.4)	
Diagnosis of hypertension				
Yes	36 (20.6)	20 (27.8)	16 (15.5)	0.038*
No	139 (79.4)	52 (72.2)	87 (84.5)	
Diagnosis of dyslipidemia				
Yes	54 (30.9)	26 (36.1)	28 (27.2)	0.138
No	121 (69.1)	46 (63.9)	75 (72.8)	
Regular physical activity				
Doing	89 (50.9)	34 (47.2)	55 (53.4)	0.258
Not doing	86 (49.1)	38 (52.8)	48 (46.6)	
Smoking				
Smoking	31 (17.7)	10 (13.9)	21 (20.4)	0.237
Used to smoke but not now	30 (17.2)	16 (22.2)	14 (13.6)	
Does not smoke	114 (65.1)	46 (63.9)	68 (66.0)	
Alcohol intake				
Yes	-	-	-	-
No	175 (100.0)	72 (100.0)	103 (100.0)	

*A chi-square test p<0.05, **p<0.01

relationship with the food insecurity level. As the food insecurity score increased, the severity of food insecurity also increased. However, the severity of food insecurity decreased as the waist/height ratio of individuals increased. The regression analysis results additionally revealed a significant negative relationship between BMI value, waist circumference and waist/height ratio and food insecurity.

It was determined that the rate of food insecurity among housewives was higher than the rate among individuals working various jobs. One of the reasons for this situation was that in most households, housewives took the

Table 3. Participants' anthropometric measurements according to their food security status

	Female (n=155)			Male (n=20)		
	Food insecure (n=64) Mean±SD	Food secure (n=91) Mean±SD	*p*	Food insecure (n=8) Mean±SD	Food secure (n=12) Mean±SD	*p*
Age (year)	40.8±10.93	37.7±11.39	0.053	44.1±16.05	37.1±14.03	0.343
Body weight (kg)	83.5±15.86	77.6±13.9	0.007**	85.5±25.73	87.3±12.75	0.999
Height (cm)	159.6±5.88	161.3±5.9	0.137	172.9±8.85	176.9±7.82	0.305
Body mass index (kg/m^2)	32.4±7.77	29.7±5.2	0.003**	28.5±8.00	27.9±3.42	0.792
Waist circumference (cm)	93.4±9.92	87.8±9.2	0.001**	93.1±13.37	93.6±7.00	0.851
Hip circumference (cm)	115.5±10.86	123.0±105.7	0.060	112.6±10.13	112.4±11.09	0.851
Lean body mass (kg)	48.8±5.91	48.2±5.59	0.217	60.1±12.94	63.8±8.04	0.571
Lean body mass (%)	58.7±7.36	62.8±7.65	0.001**	73.0±11.17	73.8±6.22	0.970
Body fat mass (kg)	36.4±12.43	29.5±9.48	0.001**	25.1±14.78	23.0±7.82	0.851
Body fat percentage (%)	40.8±6.68	37.1±5.9	0.001**	27.0±11.16	26.0±5.92	0.910
Body water content ratio	43.1±5.05	45.9±4.5	0.001**	53.5±8.20	53.9±4.18	0.970

Mann whitney-U test *p<0.05 **p<0.01; SD: Standard Deviation

Table 4. Logistic regression analysis for food insecurity

Variables	OR	95% CI	*p*
Age (year)	0.974	0.947–1.001	0.001**
Body mass index (kg/m^2)	0.942	0.897–0.987	0.001**
Waist circumference	0.949	0.915–0.984	0.001**
Waist/Height ratio	0.003	0.000–0.006	0.001**
Education status			
University	1.000		0.001**
Primary school	0.196	0.105–0.287	
Employment status			
Employed	1.000		0.001**
Housewife	0.292	0.173–0.411	

**Logistic regresion test p<0.001; OR: Odds Ratio; CI: Confidence Interval

role of food providers and babysitters, and they voluntarily prioritized the nutritional needs of other household members over their own.

Diet quality is an important risk factor for the incidence of chronic diseases. Higher HEI scores are associated with better prevention of obesity, type 2 DM and cardiovascular diseases and decreased mortality rates due to cardiometabolic diseases (Wang *et al.* 2015). Individuals experiencing food insecurity cannot easily access food groups that provide the energy and nutrients they need. The lack of dietary diversity and the inability to consume the recommended food groups (meat, milk, fruit, etc.) reduce the quality of the diet. In earlier studies, food insecurity has been associated with low HEI-2015 scores (La Mantia 2020; Landry *et al.* 2019). Similarly, among 1,568 participants, food insecurity was associated with lower diet quality (Larson *et al.* 2020). However, the cost of nutrition in each country differs. The prices of packaged and ready-to-consume meals and frozen foods, among others, are higher in Türkiye. For example, a pack of frozen potatoes or pizza costs more than a kilo of fresh vegetables (Atay-Haspolat 2020). In this context, an individual living and possibly working in a food-secure household may not have time to prepare meals, so they turn to consuming more practical ready-to-cook frozen products or ready-to-eat meals. The diet quality of individuals who are constantly fed in this way may deteriorate. This study showed that the percentage of food-secure individuals with poor diet quality was higher than that of food-insecure individuals with poor diet quality. This finding contradicts those findings in existing literature described above.

Food insecurity may differ by gender. The distribution of food insecurity by gender may also differ by geographical region. For example, a study conducted in Europe showed that women were more exposed to food insecurity than men (Grimaccia & Naccarato 2022). This study, yielded no statistically significant correlation between food insecurity and gender. Within the study cohort, males were a minority (n=20, 11.4%). More male paerticipants could have been included in the study for a more balanced genderdistirbution. Nonetheless, the low number of males accesing the study site constrained this effort.

Waist circumference is one of the most important indicators of abdominal obesity. A high waist circumference is parallel to increased risk of developing cardiometabolic diseases. In this study, a significant negative correlation was found between food insecurity and waist circumference. In Mexico, a study with women found that those without nutritional security had higher waist circumference values (Lopez-Gambino *et al.* 2020).

Waist/height ratio is a currently used method in the assessment of obesity and chronic disease risk. In a study conducted with 10,419 adults in China, after an average of 2.8 years of follow-up, waist/height ratio were found to be positively associated with diabetes risk (Fan *et al.* 2020). In another study conducted in Mexico with adults older than 20 years of age, participants with higher waist-to-height ratios had higher risk of developing dyslipidemia, hypertension and insulin resistance than participants with low waist-to-height ratios (Rangel-Baltazar *et al.* 2019). In this study, based on a regression analysis, a significant negative correlation was found between waist/height ratio and food insecurity. This shows that as the waist/height ratio increased, food insecurity would decrease. This study further revealed that the percentage of food-secure individuals with high risk according to their waist/height ratios was found to be higher than that of food-insecure individuals. Increasing waist/height ratio means increasing cardiometabolic risk. However, the results found in this study did not support the hypothesis that food insecurity may increase cardiometabolic risk.

One of the important factors affecting cardiometabolic risk is DM. Individuals with food insecurity may develop peripheral insulin resistance as a result of constant food shortages. This may increase the risk of developing DM. In this study, the percentage of food-secure individuals diagnosed with diabetes was found to be higher than that of food-insecure individuals ($p>0.05$). Individuals with food insecurity consume more processed foods with high sodium content and less amounts of certain nutrients (magnesium, vitamin C, etc.), which affects the development of HT (Helmick *et al.* 2018). In this study, the percentage of food-insecure individuals diagnosed with HT was found to be higher than that of food-secure individuals ($p<0.05$). Similar to our results, several earlier studies have found a positive relationship between food insecurity and the incidence of HT (Irving *et al.* 2014; Murillo-Castillo *et al.* 2018).

A diet high in saturated fat causes an increase in serum LDL cholesterol levels. Several works have reported findings on this (Shin *et al.* 2015; Murillo-Castillo *et al.* 2018). Shin *et al.* (2015), reported that individuals with food insecurity have lower serum HDL levels compared to those with food security. This study, the percentage of food-insecure individuals diagnosed with dyslipidemia was lower than that of food-secure individuals ($p>0.05$).

The risk of cardiometabolic disease can be reduced with regular physical activity (WHO 2020). In this study, it was found that individuals who engaged in regular physical activity were more likely to have food security ($p>0.05$). Food-insecure individuals do not spare time for regular physical activity as they spend most of their time during the day generating income or housekeeping. Furthermore, from a societal perspective, nutritionally insecure societies lack sufficient information about the importance of regular physical activity (WHO 2020).

It has been determinde that anxiety due to food insecurity triggers stress, leading individuals to smoke as coping mechanism (Twyman *et al.* 2014). In food-insecure households, smoking may suppress appetite during periods of limited food availability. Increased smoking among nutritionally insecure individuals can elevate the incidence of simiking-related bronchitis, lung cancer, and prostate cancer, as well as other diseases and health expenditures, creating a vicious cycle. Conversely, some individuals with food insecurity may quit smoking to allocate their limited budget to essential needs (Farrelly & Shafer 2017). Therefore, studies examining the effect of smoking status on food insecurity have shown conflicting results in the literature. A positive relationship between smoking rate and food insecurity (Kim-Mozeleski *et al.* 2019; Kim *et al.* 2017) or a negative relationship (Bergmans 2019) can both be observed in the literature. In this study, however, no significant relationship was found between smoking status and food insecurity.

Data collection coincided with the Covid-19 pandemic, affecting sample size and resulting in the minimum planned participant numbers being reached. Additionally, the small number of male participants, and the selection of the sample from those visiting the diet outpatient clinic are limitations.

CONCLUSION

In this study, out of the 175 adult participants, 58.9% were found to be food-secure, while 41.1% were food-insecure. Of all these participants, only 6.3% had good diet quality. The presence of hypertension, one of the cardiometabolic risk factors, was found to be associated in food-secure and food-insecure individuals. However, since this study was designed as a cross-sectional study, it cannot provide a cause-effect association. Therefore, further studies are needed for more definitive results.

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DECLARATION OF CONFLICT OF INTERESTS

The authors declare no potential conflicts of interest.

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Research Article

The Effect of Healthy Boba Pearl Drink on Post-Prandial Glucose

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ABSTRACT

This study was a randomized controlled trial using a repeated experimental design with a pre-test and a post-test control group. The study subjects were women of childbearing age, aged 20–30 years, with no diagnosed chronic diseases and with baseline fasting blood glucose levels less than 125 mg/dL. Participants were randomly assigned to treatment and control groups. The treatment group received 350 mL of healthy boba drink, while the control group received 350 mL of commercial boba drink. The healthy boba drink was made by mixing 67 mL of fresh milk, 133 mL of soy milk, 1 g of stevia sweetener, 100 g of red dragon fruit, and 40 g of healthy boba pearls. It contained 215 kcal of energy, 8.82 g of protein, 8.90 g of fat, 30.78 g of carbohydrate, and 1,808 g of fiber. Each group had an initial fasting blood glucose levels measurement before the intervention, and postprandial glucose levels were measured at the end of the intervention (one time of intervention). The collected data were analyzed univariately to analyze the effect of treatment on postprandial glucose using the independent t-test. The result indicated that the subjects in the treatment group and 96.2% of the subjects in the control group had fasting glucose levels less than 126 mg/dL. Both participants in the control and treatment groups had postprandial glucose levels less than 200 mg/dL. The mean glucose level was 89.49 mg/dL in the treatment group and 92.57 mg/dL in the control group. The study results showed that the treatment group that consumed the healthy boba drink had a lower average postprandial glucose level than the control group. The statistical test results showed that there was a significant effect of healthy boba drink consumption on postprandial glucose levels in the treatment group ($p<0.000$). It is concluded that the healthy boba drink intervention had a significant effect ($p<0.000$) on lowering blood glucose by 5.82 mg/dL after the initial treatment. The results of this study are a major first step for future work to develop a healthier boba drink.

INTRODUCTION

The prevalence of prediabetes, especially the prevalence of IGT, in the young age group (15–24 years) in Indonesia has increased drastically every year (Paramita & Lestari 2019). The term of prediabetes is significant because of its widespread occurrence and the heightened likelihood of development to overt Type 2 Diabetes Mellitus (T2DM) in individuals with prediabetes (Schlesinger *et al.* 2022). There has been a significant increase in the number of diabetes cases in Indonesia, with the reported cases increasing from 10.7 million in 2019 to 19.47 million in 2021 (International Diabetes Federation 2021). According to the 2018 Basic Health Survey (Riskesdas) in Indonesia, the rate of diabetes cases among women was 1.8% higher than among men, indicating a slight increase from 2013, when the difference was only 1.7% (MoH

RI 2013 & 2018). Inadequate management of T2DM can lead to the onset of non-communicable conditions such as cardiovascular disease, stroke and renal failure. The Indonesian government has pledged to prioritize the prevention and treatment of non-communicable diseases.

Boba, or bubble tea, is particularly popular in large urban centers with significant populations of Asian American Pacific Islander (AAPI) youth and young adults (Min *et al.* 2017). It has emerged as a significant player in the global beverage industry and has garnered considerable attention in mainstream culture. The global market value of boba tea was recorded at USD2.15 billion in 2019, increasing to USD2.3 billion in 2020. It is projected to reach USD4.3 billion by the year 2027 (Chia *et al.* 2023). Boba tea consists of whole milk, sweetened condensed milk, tea, sugar, and chewy tapioca pearls, which are usually mixed with sweet chocolate or soaked in liquid palm sugar. It has a distinctive taste and chewy sensation that can be addictive (Fajrin *et al.* 2022).

Due to its extremely high sugar content, boba tea, despite its popularity, poses a potential health risk that can cause many negative side effects. Therefore, boba milk tea should be consumed with caution as it is not the healthiest drink available (Zubairi *et al.* 2023). Several attempts have been made by researchers to find alternative solutions to the problem of T2DM, including by the development of innovative boba drink products, which are originally high in energy and fat and low in fiber. Our previous study resulted in a healthier boba drink that was low in energy and fat and high in fiber. The healthy drink with healthy boba pearls used in this study was the modification of the commercial boba tea with a ratio of tapioca flour, porang glucomannan flour and kappa carrageenan of 7:2:1 and passed an acceptability test with a relatively high acceptability level (≥75%) (Fajrin *et al.* 2022). Porang (*Amorphophallus muelleri* Blume) is an ideal dietary choice due to its high fiber content and low cholesterol levels, making it suitable for those following a diet and those with hypertension and diabetes mellitus. Moreover, porang tubers contain glucomannan, which is known for its ability to reduce blood glucose and cholesterol levels (Suryana *et al.* 2022). Therefore, a study is needed to determine whether a drink containing healthy boba pearls has an effect on increasing postprandial glucose or not.

METHODS

Design, location, and time

A randomized controlled trial study using an experimental design with a pre-and post-test control group and was conducted in Pedurungan Tengah Urban Village, Pedurungaan District, Semarang City, Central Java Province from August to October 2023. This study has received ethical approval from the Health Research Committee (KEPK) of Semarang Health Polytechnic of the Ministry of Health with reference number 1134/EA/KEPK/2023.

Sampling

In this study, 54 subjects were randomly assigned in equal numbers to the treatment and control groups by lottery. Participants who got the odd number in the lottery entered the treatment group and those who got the even numbers in the lottery entered the control group. The target population for this research was women of childbearing age in Pedurungan District, Semarang City. Samples were collected using the consecutive sampling method. The inclusion criteria for this study were healthy women of childbearing age, aged 20–30 years, with no diagnosed chronic diseases and with baseline fasting blood glucose levels less than 125 mg/dL.

Healthy boba drink preparation

The formulation used to prepare the healthy boba drink for the treatment group in this study was based on modifications of Fajrin *et al.* (2022). Healthy boba pearls were prepared using tapioca flour, porang glucomannan flour and kappa carrageenan in a ratio of 10:2:1 (a mixture of 11.25 g of tapioca flour, 2,625 g of porang glucomannan flour and 1,125 g of kappa carrageenan). The boba pearls were boiled in boiling water for 25 minutes. The drink was made by mixing 67 mL of fresh milk, 133 mL of soy milk, 1 g of stevia sweetener, and 100 g of red dragon fruits until homogenized, adding 40 g of healthy boba pearls, and packaged in a 350 mL plastic cup. Referring to laboratory analysis of previous study by Fajrin *et al.* (2022), the drink with healthy boba pearls for the treatment group contained 215 kcal of energy, 8.82 g of protein, 8.90 g of fat, 30.78 of carbohydrate and 1,808 g of fiber.

The placebo beverage for the control group was prepared by mixing 25 g of instant milk tea, 200 mL of water, 20 mL of evaporated milk, and 10 g of palm sugar, and adding 40 g of boiled instant boba pearls, then packaged in a 350 mL plastic cup. According to the laboratory analysis of the previous study by Fajrin *et al.* (2022), the placebo beverage contained 215 kcal of energy, 0.66 g of protein, 2.34 g of fat, 68.69 g of carbohydrate and 0 g of fiber.

Data collection

The first step in data collection was to screen the population based on predetermined subject criteria. Participants who met the inclusion criteria were divided into treatment and control groups. All subjects had an initial fasting blood glucose levels measurement before the intervention and then then their post-prandial blood glucose levels were measured again two hours after the intervention. The treatment group received 350 mL of healthy boba drink, while the control group received 350 mL of commercial boba drink.

Fasting blood glucose and postprandial glucose were determined by blood tests performed at baseline and at the end of the intervention. Participants were instructed to visit an established and registered clinical laboratory to undergo these tests and have their blood glucose levels assessed. A trained technician at the laboratory collected blood samples by venipuncture. Biochemical analysis was performed using the Indiko™ Plus Clinical Chemistry Analyzer. The results of these clinical assessments were then e-mailed to the researchers. Data on subject characteristics (medical history, nutritional status, physical activity, and dietary intake) were obtained by completing a questionnaire, while macronutrient and fiber intake during the last 24 hours before treatment was obtained by direct interview using the 24-hour recall method. The nutritional status of the participants was determined by measuring body weight and height.

Data analysis

Categorical variables (medical history, nutritional status, physical activity, and medication use) obtained are presented in the form of proportions, while numerical variables (blood glucose in the treatment group and control group before and after given treatment) are presented in the form of mean, median, standard deviation, and minimum-maximum values. Data on nutritional status, macronutrient intake, and fiber intake of subjects in the treatment and control groups were tested using independent t-test. The confidence interval and significance level for the independent t-tests were set at 95% and 0.05, respectively.

RESULTS AND DISCUSSION

Fifty-four subjects were screened, but only 52 subjects met the inclusion criteria. Two subjects were excluded from the control group because they had fasting blood glucose greater than 125 mg/dL, so the number of subjects in the treatment group was 27 and in the control group was 25 (Table 1). The subjects were evenly distributed and there were no significant differences between the two groups.

Characteristics of blood glucose levels

The results of the examination of blood glucose levels for both groups are presented in Table 2. All subjects in the treatment group and 100% of subjects in the control group had fasting glucose levels less than 126 mg/dL. Both participants in the control and treatment groups had postprandial glucose levels less than 200 mg/dL. The mean fasting glucose level was 89.49 mg/dL in the treatment group and 91.12 mg/dL in the control group.

Body mass index

The average BMI in the treatment group was 22.39 kg/m^2 and in the control group was 22.45 kg/m^2 with the category of normal nutritional status. There was no difference in BMI between the two groups (p>0.000).

Macronutrient and fiber intake

Macronutrient and fiber intake before treatment was collected by interview using a 24-hour recall method. Table 3 shows the intake of macronutrients and fiber in both groups. The mean energy intake for the treatment group was 1,498 kcal and for the control group was 1,455 kcal. Energy adequacy based on the Recommended Dietary Allowances (RDA) means that the energy requirement for women between 20 and 30 years of age is 2,250 kcal. In terms of energy adequacy based on the RDA, both the treatment and control groups met about 65–67% of the RDA. The protein intake of the treatment group was 58.6 g and that of the control group was 56.0 g. The

Table 1. Characteristics of subjects

Variable	Treatment group (n=27)	Control group (n=25)	*p*
	n (%)	n (%)	
History of nutritional counselling			
Yes	11 (40.7)	11(44)	
No	16 (59.3)	14 (56)	
History of Diabetes			
Yes	4 (14.8)	3 (12)	
No	23 (85.2)	22 (88)	
Frequency of exercise			
<3 times/week	25 (92.6)	25 (100)	
≥3 times/week	2 (7.4)	0 (0)	
Nutritional status (by BMI)	22.38±3.56	22.30±3.36	0.931
Underweight	2 (7.4)	4 (16)	
Normal	14 (51.9)	11 (44)	
Overweight	7 (25.9)	4 (16)	
Obesity	4 (14.8)	6 (24)	

BMI: Body Mass Index

protein adequacy based on the RDA is 60 g, so the protein intake of the treatment and control groups was 93–97%.

Effect of healthy boba pearl drink consumption on postprandial glucose levels

The results of the study on the effect of consumption of the drink containing boba pearls on postprandial glucose levels in the control and treatment groups are presented in Table 4. The results reported that the treatment group that consumed healthy boba drink had a lower average postprandial glucose level than the control group. The statistical test results revealed that there was a significant effect of healthy boba drink consumption on postprandial glucose levels in the treatment group (p<0.000).

Boba drink is a type of modern drink that has emerged among Indonesians in recent years. It is a drink made of black tea mixed with fruit and milk flavors, and topped with small, chewy, dark tapioca pearls called boba, which is usually drizzled with brown sugar. Boba milk tea is served with high-calorie sugar, with sugar content ranging from 38 to 96 g of granulated sugar and energy content ranging from 299 to 515 kcal, depending on size and additional toppings. Moreover, some boba drinks contain additional sugar in the form of sweeteners such as High Fructose Corn Syrup (HFCS) or sucrose, which are commonly found in Sugar-Sweetened Beverages (SSBs). High Fructose Corn Syrup (HFCS) is particularly 55% fructose and 45% glucose, while sucrose is 50% fructose and 50% glucose. Increased intake of added sugars is the leading cause of metabolic diseases, such as type 2 diabetes. Unfortunately, sweetened drinks are high in sugar, so they are high in calories, but they reduce satiety and have low nutritional value (Veronica & Ilmi 2020).

Porang, or Amorphophallus muelleri Blume, has tubers that contain glucomannan,

Table 2. Characteristics of blood glucose levels

Glucose levels (mg/dL)	Treatment group (n=27)	Control group (n=25)	*p*
	Mean±SD	Mean±SD	
Fasting glucose levels	89.49±5.12	91.12±7.44	0.000
Postprandial glucose levels	83.67±4.89	94.40±17.84	0.000

SD: Standard Deviation

Table 3. Characteristics of macronutrient and fiber intake

Intake	Treatment group (n=27)		Control group (n=25)		p
	Mean±SD	Min–Max	Mean±SD	Min–Max	
Energy (kcal)	1,498±413.8	980–2,559	1,437±413.8	918.40–2,410.6	0.581
Protein (g)	58.6±24.9	7.2–107.5	56.0±22.9	22.80–134.78	0.740
Fat (g)	54.5±21.9	21.4–111.4	52.5±28.6	21.70–160.20	0.595
Carbohydrates (g)	170.8±73.6	24.6–377.1	177.3±58.8	85.00–344.10	0.784
Fiber (g)	7.02±3.46	1.1–12.3	4.7±2.7	0.10–13.10	0.012*

SD: Standard Deviation; Min: Minimum; Max: Maximum

Table 4. Postprandial glucose levels after consuming boba

Glucose levels (mg/dL)	Treatment group (n=27)	Control group (n=25)	p
	Mean±SD	Mean±SD	
Fasting glucose levels - Before intervention	89.49±5.12	91.12±7.44	0.000
Postprandial glucose levels - After intervention	83.67±4.89	94.40±17.84	0.000
p	0.000	0.380	

SD: Standard Deviation

a water-soluble fiber that can lower blood cholesterol and blood glucose levels and reduce body weight (Nissa & Madjid 2016). This fiber has health benefits and can serve as a dietary replacement for individuals dealing with obesity, type 2 diabetes, and dyslipidemia. Porang flour, a low-fat, high-fiber food ingredient, contains 64.98% glucomannan, 2.5% dietary fiber, and 0.02% fat (Thelmalina & Wirasuta 2023; Mahirdini & Afifah 2016; Fatchiyah *et al.* 2019). Glucomannan, a highly soluble dietary fiber with a dry matter content of 15–64%, could lower blood glucose levels by forming a thick gel that slows gastric emptying in the small intestine. This fiber fermentation, facilitated by the colonic microbiome, stimulates the production of GLP-1, an incretin hormone (Thelmalina & Wirasuta 2023).

Soy is a low-glycemic food ingredient with isoflavones, dietary fiber and antioxidant properties. It helps control blood glucose levels, protects pancreatic cells from oxidation, and regenerates damaged cells. Soy milk has been shown to help control blood glucose levels in people with T2DM (Pramono *et al.* 2020; Yulifianti *et al.* 2018). Soy protein contains glycine and arginine, amino acids that stimulate pancreatic beta cells to regulate blood glucose levels. These amino acids increase insulin secretion and glucose transport to the body's cells. The main isoflavones, genistein and daidzein, act as antioxidants and inhibitors of enzymes that prevent rapid glucose absorption. However, some studies have suggested that the effect of soy isoflavones on glucose levels is not statistically significant (Pertiwi & Murbawani 2012; Barańska 2021). Red dragon fruit could be used as a substitute food to lower blood glucose levels and inhibits oxidative reactions because it contains high levels of vitamins and antioxidants. Its flavonoid content prevents damage to beta-cells and increases insulin sensitivity. The fruit's fiber binds water, reducing glucose levels and triggering insulin production. Several studies showed that red dragon fruit was effective in reducing fasting blood glucose levels in individuals with overnutrition (Ayuni 2020; Fadlilah *et al.* 2020).

The results of the reduction of postprandial glucose levels in this study are presented in Table 4. There was a significant effect of consuming healthy boba drink on postprandial glucose levels in the treatment group ($p<0.000$), as indicated by the mean fasting glucose level of the treatment group being 89.49 mg/dL, and the mean postprandial glucose level after consuming healthy boba drink being 83.67 mg/dL, so there was a decrease in blood glucose by 5.82 mg/dL. This is directly proportional to the study results of Thelmalina and Wirasuta (2023), which found the average initial fasting blood glucose level in the treatment group that received a high fructose diet and 200 mg of porang flour. The results showed that there was a decrease of 176 mg/dL (57.7%)

in mice given a high fructose diet and 200 mg porang flour group, which was a quite significant decrease in blood glucose levels (Thelmalina & Wirasuta 2023). A study conducted by Laboro *et al.* (2023) proved that the administration of 200 mL of soy milk for seven days resulted in the reduction of blood glucose in diabetic patients in the treatment group, as evidenced by the results of the average blood glucose in the treatment group before the initial treatment from 213.30 mg/dL to 105.40 mg/dL after the initial treatment (Laboro *et al.* 2023). Apart from that, a study on dragon fruit conducted by Nisa *et al.* (2021) found that administration of dragon fruit juice for seven days resulted in significant changes in blood glucose levels in people with diabetes mellitus (p=0.026), where the average blood glucose level before treatment was 305.7 mg/dL and decreased to 241.7 mg/dL after treatment. It is also known that 9 out of 15 subjects (60%) had normal blood glucose levels after consuming dragon fruit for seven days (Nisa *et al.* 2021).

For people with diabetes mellitus, it is recommended to consume 20 g–35 g of fiber per day, with a minimum recommendation of 25 g per day. Consumption of less than 25 g of fiber per day is considered low fiber intake, while consumption of 25 g or more of fiber per day is considered adequate fiber intake (Soviana & Maenasari 2019). An analysis of 11 pooled RCTs (13 comparisons) with a minimum 8-weeks duration examined the effect of dietary fiber on glycemic control in T2DM patients. The inclusion of fiber-rich foods or fiber supplements in the diet resulted in a significant 0.55% reduction in HbA1c levels, equivalent to an average reduction of 4.75%. In addition, fasting plasma glucose levels lowered by 10 mg/dL. Soluble fiber's physiological effects include modulation of the postprandial glycemic response through gastric and small bowel functions. These effects include delayed gastric emptying, altered gastrointestinal myoelectric activity, delayed small bowel transit, decreased glucose diffusion through the unstirred water layer, and decreased α-amylase accessibility to substrates due to increased gut viscosity (Sillva *et al.* 2013). The reduction in blood glucose levels in this current study is related to the use of alternative food ingredients to make a healthy boba drink to reduce blood glucose levels, which contains fiber per serving of 1.808 g (9.04% of the daily fiber requirement).

CONCLUSION

The results of this study showed that the administration of healthy boba pearl drink to the treatment group resulted in a significant reduction in postprandial glucose levels of the group (p<0.000). The average fasting glucose levels of the treatment group before the intervention was 89.49 mg/dL, and after the administration of healthy boba pearl drink, the average postprandial glucose level decreased to 83.67 mg/dL. There was a 5.82 mg/dL decrease in postprandial glucose levels after the initial treatment.

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DECLARATION OF CONFLICT OF INTERESTS

Authors declares no conflicts of interests.

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Research Protocol Article

Effectiveness of *Cosmos caudatus* Extract in Improving Health-Related Parameters among Older Adults with Sarcopenia: A Study Protocol

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ABSTRACT

This is a randomized, two-arm, double-blind, placebo-controlled study that will be performed among older adults in Kelantan, Malaysia, for 12 weeks. This study aims to determine the effectiveness of a 12-week *Cosmos caudatus* (*C. caudatus*) extract supplementation on dietary intake, cognition, mental health, sleep quality, disability, gut microbiota, physical activity, frailty, metabolites, and protein synthesis pathways among older adults with sarcopenia. This study will involve 64 older adults diagnosed with sarcopenia that will be enrolled and randomly allocated with 1:1 ratio, where 32 subjects required in both intervention and placebo group. The intervention group will receive 500 mg/day of *C. caudatus* supplementation, while the placebo group will be given 500 mg/day of maltodextrin. Study outcomes including sarcopenia status, dementia, motoric cognitive risk, dietary pattern, anthropometric data, physical fitness, mental health, disability and sleep quality will be measured. Blood will be taken for analysis of protein synthesis pathways (using blood plasma) and untargeted metabolomics at baseline and the 12th week. Fecal sample will be collected from a subsample of 24 subjects for gut microbiota analysis. This study is one of the pioneers randomized controlled trials to evaluate *C. caudatus* efficacy on various health related outcomes among sarcopenic community-dwelling elderly. This study findings are hoped to have the potential to improve health awareness of *ulam* or fresh salad consumption, specifically *C. caudatus* and prove its effectiveness as a nutraceutical product for older adults with sarcopenia. The Australian New Zealand Clinical Trials Registry (ANZCTR) has this trial listed as registered with code (ACTRN12623000046606p).

INTRODUCTION

In Southeast Asia, the aging population has been expanding, with the proportion of individuals 65 years of age and older increasing from 6% to 11% between the years 1990 and 2019 (You *et al.* 2021). This phenomenon has caused a rise in the number of elderly with disabilities and dependency that is related to sarcopenia. Sarcopenia is an age-related condition characterized by a gradual loss of muscle mass and muscle function in the skeletal system (Papadopoulou 2020). Over 50 million older adults currently suffer from sarcopenia, and over the next 40 years, the figure is projected to rise to 200 million (Ramoo *et al.* 2022). Sarcopenia might cause other geriatric syndromes, including poor mental health, frailty, and, to some extent, significant disabilities (Gao *et al.* 2021; Aurelia *et al.* 2015; Ramoo *et al.* 2022).

To date, some of the most effective treatments for sarcopenia are nutritional therapies, physical activity, and androgen therapy, apart from other behavioral and pharmacological strategies (Aurelia *et al.* 2015). *Ulam* which is mostly made up of fresh leaves of medicinal plants that have numerous therapeutic properties, has been widely used, but studies on the development of *ulam* as a nutraceutical product are still limited (You *et al.* 2018). One of the notable *ulams* used as nutraceutical products is *Cosmos caudatus* (*C. caudatus*), referred to as *ulam raja* in the local tongue (You *et al.* 2021). *C. caudatus* possesses a variety of pharmacological properties, such as antidiabetic, antibacterial, antioxidant, antihypertensive, antihyperlipidemic, and antiosteoporosis (Moshawih *et al.* 2017). *C. caudatus* ethanolic extract is able to inhibit α-glucosidase (Ahda *et al.* 2023). *C. caudatus* methanolic extract is effective in inhibiting the growth of certain microorganisms, including E. coli and S. aureus, in oyster mushrooms (Yusoff *et al.* 2015). An aqueous extract of *C. caudatus* is effective in decreasing the heartbeat and amplitude of stroke volume induced by adrenaline (Cheng *et al.* 2015).

Most studies regarding the use of *C. caudatus* are pre-clinical animal studies, but the clinical effect of *C. caudatus* in humans is still obscure. Human clinical studies using *C. caudatus* demonstrated that supplementation with *C. caudatus* improves insulin sensitivity, C-Reactive Protein (CRP), and serum metabolite levels of branched-chain amino acids (Cheng *et al.* 2016). You *et al.* (2021) discovered the efficacy of *C. caudatus* in improving global cognition, overall mood disturbance, tension, and oxidative stress among the elderly with mild cognitive impairment. To date, no studies have been done on *C. caudatus* being used as an intervention for sarcopenic older adults. Thus, the purpose of this study is to determine the effectiveness of a 12-week *C. caudatus* extract supplementation on dietary intake, cognition, mental health, sleep quality, disability, gut microbiota, physical activity, frailty, metabolites, and protein synthesis pathways among older adults with sarcopenia.

METHODS

Design, location, and time

This study is a double-blind placebo-controlled randomized trial for a period of 12 weeks. Table 1 shows the subjects' schedule for their enrollment, interventions, assessments, and time of visits. This research will involve sarcopenic older adults who live in five districts in Kelantan, Malaysia, including Machang, Kota Bharu, Pasir Mas, Tumpat, and Bachok. Subjects were recruited in February 2024.

The Human Research Ethics Committee (JEPeM) at Universiti Sains Malaysia has approved this study protocol (USM/JEPeM/22080543). This research will be performed according to the Declaration of Helsinki. Subjects will be required to sign an informed consent form before participating in this study. Once they were enrolled, they will be briefed on the participant information sheet.

Sampling

The sample size was calculated using the formula by Chan (2003). Thirty-two subjects will be recruited for each treatment and control group with a post-intervention standard deviation of 0.37 meters per second, 80% power, and a 95% confidence interval with an additional drop-out rate of 20%. The mean and standard deviation calculations were based on the research by Kim *et al.* (2012) that evaluated the effect of nutrition and exercise intervention in improving walking speed among community-dwelling sarcopenic in older adults over a three-month period.

The sampling method used for screening is called convenient sampling. After the researcher obtained the number of subjects with sarcopenia, the scouting procedure will be done for a randomized controlled trial. A 1:1 allocation ratio will be used to randomly assign subjects to the intervention or control groups. The randomization sequence of the subjects will be generated using a randomization website (https://www.randomization.com) by simple randomization.

Inclusion criteria. Subjects aged 60 years and older diagnosed with sarcopenia in accordance with the 2019 guidelines of the Asian Working Group for Sarcopenia (AWGS) (only meeting two criteria: low muscle mass and poor muscle strength or poor physical performance), non-smoking, not taking any other vitamins or herbal or traditional medications, and not consuming fruits or vegetables or not meeting the recommended intake of two servings of fruits and at least three servings of vegetables will be recruited.

Exclusion criteria. Older adults who are undergoing regular hemodialysis, bedridden, hospitalized, or living in a long-term care facility, have severe sarcopenia (meeting all the three criteria of AWGS 2019 namely poor muscle strength, low muscle quantity, and poor physical performance), gastrointestinal surgery or gastrointestinal radiation therapy or chemotherapy, chronic kidney diseases, diarrhoea, chronic constipation or gastrointestinal diseases (inflammatory bowel disease, irritable bowel syndrome, haemorrhoid, diverticulitis), dementia, and also those who are on tube feeding or antibiotics for the past 30 days, corticosteroid, immunosuppressants, warfarin therapy or on medications affecting intestinal motility such as laxatives, antidepressants, opioid, anticholinergic, prebiotic and probiotic during the study period will be excluded.

Intervention. The treatment group will receive 500 mg/day of *C. caudatus* extract (two capsules per day of size 0), while the control group will receive a similar dose of maltodextrin daily for 12 weeks. The dosage of 500 mg/day is based on prior research by You *et al.* (2021). Subjects with mild cognitive impairment were given 500 mg of *C. caudatus* supplement for 12 weeks, and no adverse effects were reported.

Patient safety. Subjects will be asked if they experienced any side effects such as diarrhea, flatulence, headache, or rashes following the consumption of *C. caudatus*. These side effects are rarely observed, but if suspected, treatment needs to be stopped and reported as an adverse outcome. Proper medical treatment will be given to the subjects who claimed to experience side effects after consuming *C. caudatus* supplementation. Subjects who are non-compliant with the study procedure, such as not consuming the given amount of *C. caudatus* for more than 20% of the study duration or experiencing side effects, were excluded from the study.

Adherence. Weekly telephone calls will be made to monitor adherence and address subjects' concerns, such as the presence of any side effects after consuming *C. caudatus* supplementation. Compliance will be monitored by the researcher by asking the subjects to return the supplement bottle every month. Moreover, the subjects will be given a small diary with a medication chart and will be required to mark the chart daily after consuming the supplements.

Study outcome. Table 2 shows the study outcomes including primary outcome and secondary outcome.

Data collection

Subjects from both groups will be required to present for data collection at three different points: baseline, 6th week, and 12th week. Data that will be collected include sociodemographic profile, blood pressure, anthropometry, physical fitness, dietary intake, assessment of sarcopenia, motoric cognitive risk syndrome, mental health, sleep quality, disability, frailty (physical, cognitive, and social), fecal collection, and blood withdrawal. Only blood withdrawal and fecal collection which will be done twice, at baseline and the 12th week.

The subjects will be asked regarding their socio-demographic profiles, which include questions regarding their age, sex, household income, employment status (current and past), marital status, education level, living arrangement, and smoking status. Medical history includes the current and past comorbidities experienced by individuals or any surgical procedure done in the past year. Medication or dietary supplement intake will be recorded. An Omron digital blood pressure monitor will be used to measure blood pressure. Anthropometry will include measurements of height, weight, and calf circumference. The Karada Scan Omron Body Composition Monitor HBF-214 by Omron Healthcare, Kyoto, Japan, will be used to measure weight. A Seca North America, Chino, USA, stadiometer will be used to measure height. For older adults with scoliosis, height is measured using arm span, which is the separation between the middle tips of two fingers. For height estimation using arm span, the formula by Shahar and Pooy (2003) will be used for height calculation. Calf circumference is the measurement of the broadest part of the calf. It is taken when the subject is sitting with his feet touching the ground. Two readings will be taken for the anthropometric measurements, and the analysis will utilize the mean of the two readings.

Assessment of sarcopenia. Sarcopenia will be diagnosed using the AWGS (2019) guideline. In this study, only subjects with sarcopenia will be selected, and those with severe sarcopenia is excluded. Those with sarcopenia will have the following criteria:

Low skeletal muscle mass (Skeletal muscle index=M<7 kg/m^2, W:<5.7 kg/m^2)
AND Poor muscle strength (handgrip strength=M:<28 kg, W:<18 kg)
OR Poor physical performance (SPPB score ≤9)

Chen *et al.* (2020)

Meanwhile, severe sarcopenia is indicated by meeting all criteria, including low skeletal

Table 1. Time schedule for subjects' enrolment, interventions, assessments, and time visits

Timepoint	Study period				
	Enrolment	Allocation	Post-allocation (Intervention)		
			Baseline	6th week	12th week
	Jan 2024	Feb 2024	Mar 2024	Apr 2024	May 2024
Enrolment					
Eligibility screen	X	-	-	-	-
Informed consent	X	-	-	-	-
Allocation	-	X	-	-	-
Interventions					
500 mg/day of Cosmos caudatus extract	-	-	———	———	———
Assessments					
Dietary intake	-	-	X	X	X
MCR syndrome	-	-	X	X	X
Mental health	-	-	X	X	X
Sleep quality	-	-	X	X	X
Disability	-	-	X	X	X
Frailty	-	-	X	X	X
Physical fitness test	-	-	X	X	X
Blood withdrawal	-	-	X	-	X
Fecal collection	-	-	X	-	X

MCR: Motoric Cognitive Risk

muscle mass, poor muscle strength, and poor physical performance (Chen *et al.* 2020). Hand grip strength will determine muscle strength, which will be assessed using a hand dynamometer by Fabrication Enterprises Inc., New York, USA. Poor handgrip strength is determined by a value of less than 28 kg for men and less than 18 kg for women (Chen *et al.* 2020). The Short Physical Performance Battery (SPPB) will be used to assess muscle performance, which includes three major assessments: gait speed, balance tests, and a chair stand test. Poor physical performance is indicated by a total score of nine or below (Chen *et al.* 2020). Low muscle mass will be assessed using the Karada Scan Omron Body Composition Monitor HBF-214 by Omron Healthcare, Kyoto, Japan. It is indicated by appendicular Skeletal Muscle Index (SMI) values of less than 7 kg/m² for men and less than 5.7 kg/m² for women (Chen *et al.* 2020).

Assessment of dementia. Dementia will be assessed using the Montreal Cognitive Assessment-Bahasa Malaysia (MoCA-BM) with a Cronbach's α coefficient of 0.691 (Razali *et al.* 2014). A higher score denotes better cognitive ability. The score runs from 0 to 30. Subjects with scores of 22 and below will be at risk of having dementia (Mohamed Fuad *et al.* 2020).

Assessment of dietary intake. Subjects' habitual dietary consumption will be evaluated using a validated Diet History Questionnaire (DHQ). To assess dietary patterns, food items will be divided into 14 groups according to their commonalities, or references to the study by Fakhruddin *et al.* (2019). For diet quality, it will be categorized according to the Standardized Malaysian Healthy Eating Index (S-MHEI). A maximum score of 100 can be achieved by combining the elements of the new S-MHEI, which uses a density standard that is less restrictive as it focuses on an individual's nutrient intake (Jailani *et al.* 2021). Table 3 shows the components of S-MHEI.

Assessment of Motoric Cognitive Risk (MCR) syndrome. Verghese (2013) first introduced the concept of MCR syndrome, a high-risk clinical syndrome that has excellent predictive value for dementia. If any subject fulfills all criteria listed in Table 4, they will be identified as having MCR syndrome.

Assessment of mental health. The General Health Questionnaire (GHQ-12), which has been validated in Malay and has a Cronbach's α coefficient of 0.85, will be used to evaluate mental health (Ibrahim *et al.* 2014). The overall score is between 0 and 12, with 12 items. Impaired mental health is indicated by a higher score. The optimum cut-off point for GHQ-12 is 75% of the total score, which is 9.

Assessment of sleep quality. The Pittsburgh Sleep Quality Index (PSQI-M), which has been validated in Malay and has a Cronbach's α coefficient of 0.74 (Farah *et al.* 2019), will be used to evaluate the quality of sleep. The overall score is between 0 and 21. Those who receive a total score higher than five are deemed to have poor quality sleep.

Assessment of disability. Disability will be evaluated using the World Health Organization Disability Assessment Schedule (WHODAS-12). Total score ranging from 0 to 48. A cut-off score of 16 is considered an indicator of disability as it represents the 95th percentile of impairment based on WHO normative data (Mayrink *et al.* 2018).

Assessment of frailty. Frailty can be divided into a few aspects, which are physical frailty, cognitive frailty, and social frailty: a). Physical frailty: Will be determined using the Fried physical phenotype. These are the cut-off points. The phenotypes are shown in Table 5;

Positive for ≥3 frailty phenotypes=Physically frail

Positive with 1 or 2 phenotypes=Pre-fail

None=Robust

(Liu *et al.* 2020)

b). Cognitive frailty: Will be assessed based on the three criteria below: 1). Physical frailty: The subject met at least 3 criteria from the Fried physical phenotype indicating physical frailty; 2). Cognitive impairment: Assessed using MoCA as mentioned in the dementia assessment, but with a cut-off point for cognitive impairment of < 26 (Razali *et al.* 2014); 3). Functional limitation: Assessed using Instrumental Activities of Daily Living (IADL) in the Malay version. The scoring and details were explained in the MCR syndrome assessment; c). Social frailty: Will be assessed using a social frailty questionnaire developed by Makizako *et al.* (2015). There are five items in this questionnaire. Subjects will be robust if the score is 0, pre-social frailty if 1, and social frailty if 2–5 (Makizako *et al.* 2015).

Physical fitness test. Physical fitness comprises a back scratch test, a 2-minute step test, a chair sit and reach test, and a Borg scale: a). Back scratch test will be used to assess the flexibility of the upper body in the shoulder joint and shoulder arch on both the left and right sides (Seiler *et al.* 2016); b). 2-minute step test will be done to evaluate aerobic capacity, thus evaluating the subjects' level of functional fitness (Bohannon & Crouch 2019); c). Chair sit-and-reach test will be done to evaluate the flexibility of the lower body (Mayorga-Vega *et al.* 2014); d). Borg Rating of Perceived Exertion (RPE) scale is an instrument used to measure a person's effort and exertion, dyspnea, and exhaustion during physical labor (Williams 2017).

Gut microbiota analysis. Fecal samples will be collected to determine the quantity of gut microbiota. Gut microbiota analysis will be conducted on a subsample of 24 subjects (12 from the treatment group and 12 from the control group).

Blood withdrawal. A total of 6 mL of fasting blood will be obtained by venepuncture of the median cubital vein into serum separator tubes by a trained phlebotomist from Pathlab. The blood samples collected will be centrifuged in the Pathlab laboratory to separate the serum and plasma. Blood plasma will be used for Western blot analysis to detect changes in the IGF-Akt-mTOR pathway. This analysis will be conducted in the Molecular Biology Laboratory of the School of Health Sciences, Universiti Sains Malaysia. The Western Blot analysis will be done according to the adapted protocol by Singh *et al.* (2021) and Jaleel *et al.* (2005). The blood serum collected will be used for the untargeted metabolomics analysis using Nuclear Magnetic Resonance (H-NMR) spectroscopy. This will be done based on the papers by Mostafa *et al.* (2017).

Data analysis

Statistical Package for the Social Sciences (SPSS) (SPSS Inc., Chicago, USA) version 26 will be used to analyze the data. Categorical data will be shown in percentage, whereas continuous data will be shown in Mean±SD. The normality test will be done using the Shapiro-Wilk test prior to univariate analysis. An independent t-test will be used for baseline analysis to compare the study outcomes of both the treatment group and the control group. The effectiveness of the *C. caudatus* supplementation will be assessed using a two-way repeated measure ANOVA adjusted for possible confounding factors. Intention-To-Treat (ITT) analysis will be used in this investigation, meaning that all subjects will be included in the analysis regardless of whether they follow the treatment plan or finish the study, which will help to minimize selection bias. The significance level (α) will be set at 0.05, while the confidence level is at 95% for the test, and the significant p-value will be considered at <0.05.

DISCUSSION

Sarcopenia is a disease associated with aging that is usually not diagnosed in clinical practice, even when there is substantial overlap of the sarcopenia phenotype degree with comorbidities that share the same associated risks and health effects as sarcopenia (Qaisar *et al.* 2021). Therefore, the purpose of this study is to examine the factors that are related to sarcopenia, including dietary intake, cognition, mental health, sleep quality, disability, gut microbiota, physical activity, frailty, metabolites, and protein synthesis pathways. This study is the first to look at the effects of *C. caudatus* on various health-related parameters among sarcopenic older adults. Even though there is limited evidence regarding the effectiveness of *C. caudatus* as a therapy for

Table 2. Study outcomes

Category	Expectation
Primary outcome	
1. Changes in sarcopenia parameters	The consumption of *C. caudatus* supplementation for 12 weeks will improve older adults' muscle mass, muscle strength and physical performance.
Secondary outcome	
1. Changes in dietary intake	The consumption of *C. caudatus* supplementation for 12 weeks will improve older adults' overall nutrient intake, dietary pattern and S-MHEI score.
2. Changes in motoric cognitive risk syndrome	The consumption of *C. caudatus* supplementation for 12 weeks will reduce the criteria of MCR syndrome that they initially met.
3. Changes in mental health	The consumption of *C. caudatus* supplementation for 12 weeks will reduce the GHQ-12 score among older adults.
4. Changes in sleep quality	The consumption of *C. caudatus* supplementation for 12 weeks will reduce the PSQI score among older adults.
5. Changes in disability	The consumption of *C. caudatus*supplementation for 12 weeks will reduce the WHODAS score among older adults.
6. Changes in gut microbiota	The consumption of *C. caudatus* supplementation for 12 weeks will improve older adults' microbial composition (increased beneficial bacteria and decreased harmful bacteria) and improve microbial diversity.
7. Changes in physical fitness	The consumption of *C. caudatus* supplementation for 12 weeks will increase the back scratch test, 2-minute step test, chair sit and reach test, and Borg scale score among older adults.
8. Changes in frailty	The consumption of *C. caudatus* supplementation for 12 weeks will reduce the criteria of physical, cognitive and social frailty that they initially met.
9. Changes in metabolites pathways	The consumption of *C. caudatus* supplementation for 12 weeks will improve older adults' beneficial metabolic pathways such as glycolysis, oxidative phosphorylation, enhanced synthesis and utilization of amino acids, and better lipid processing.
10. Changes in IGF1-AKT-mTOR pathway	The consumption of *C. caudatus* supplementation for 12 weeks will give significant differences in protein expression (increase IGF-1, AKT Phosphorylation, mTOR Phosphorylation and their downstream targets) to indicate pathway activation.

AKT: Protein Kinase B; GHQ-12: General Health Questionnaire; IGF-1: Insulin-like Growth Factor 1; MCR: Motoric Cognitive Risk; mTOR: Mammalian Target of Rapamycin; PSQI: Pittsburgh Sleep Quality Index; S-MHEI: Standardized Malaysian Healthy Eating Index; WHODAS: World Health Organization Disability Assessment Schedule

Table 3. Standardized Malaysian Healthy Eating Index (S-MHEI) components and scoring

Dietary components	Type	Min. Score criteria	Max. Score criteria
Total grains	Adequacy	0 serving/ 1,000 kcal	1.4 servings/ 1,000 kcal
Whole grains			0.7 servings/ 1,000 kcal
Fruits			0.9 servings/ 1,000 kcal
Vegetables			1.2 servings/ 1,000 kcal
Fish			0.4 servings/ 1,000 kcal
Meat, Poultry, and Eggs			0.4 servings/ 1,000 kcal
Legumes and Nuts			0.4 servings/ 1,000 kcal
Milk and Milk Products			0.9 servings/ 1,000 kcal
Total Fat	Optimal	0–≥55% TEI	25–30% of TEI
Added Sugar	Moderation	≥25% TEI	≤5 % of TEI
Sodium		≥2,300 mg	<1,925.0 mg

TEI: Total Energy Intake; Adapted from Jailani *et al.* (2021)

Table 4. Motoric Cognitive Risk (MCR) syndrome criteria

Criteria	Explanation
Absence of dementia	Assessed using MoCA as mentioned in dementia assessment
SCD	Assessed using item 10 on GDS-15. If the answer is 'Yes', this indicates that the subject has subjective cognitive decline
Slow gait	Measured by having the subjects walk back and forth at their normal rate across a 6-meter course. A slow gait will be 1 standard deviation slower than the population's mean gait.
Preserved activities of daily living	Determined using IADL in Malay version questionnaire with Cronbach's α coefficient of 0.838 (Kadar *et al.* 2018). Higher score indicates higher functionality and lower dependency

GDS-15: Geriatric Depression Scale; IADL: Instrumental Activities of Daily Living; SCD: Subjective Cognitive Decline

sarcopenia, *C. caudatus* is chosen due to its phytochemical contents and benefits for muscle health. A prior study found that *C. caudatus* contained many flavonoids, including quercetin and catechin (Ahda *et al.* 2023). A previous study found that quercetin inhibits inflammatory receptors and their signaling pathway to decrease skeletal muscle atrophy, which might be important to prevent sarcopenia (Le *et al.* 2014). Non-flavonoid chemicals found in *C. caudatus* consist of phenolic acids, which have been shown to benefit muscles by stimulating muscle growth and/or decreasing muscular atrophy, as well as improving mitochondrial quality and lowering oxidative stress and inflammation (Nikawa *et al.* 2021).

In conclusion, the findings from this study will be able to prove the effect of *C. caudatus* on acting as a nutraceutical product for older adults with sarcopenia. The scientific data from this research could raise public health awareness of *ulam* intake, specifically *C. caudatus*, which could contribute to a further decrease in the risk of sarcopenia, a more active lifestyle, a better quality of life, and subsequently reduce the cost of healthcare, especially among older adults.

CONSENT FOR PUBLICATION

The subjects will be explained regarding the publication of their details and needed to sign the informed consent form before participating in this research. Every form is anonymous and will be input into SPSS software. The data obtained will remains confidential. Only members of the research team will have access. The data will not

Table 5. Physical frailty phenotype

Phenotype	Explanation
Unintentional weight loss	Greater than or equal to 4.5 kg or 5% of body weight in the last one year will be asked to subject
Exhaustion	Assessed by asking 2 questions from the CES-D scale which are ‘I felt that everything I did was an effort’ and ‘I could not get going’. Then, the frequency of how the subject felt as such would also be asked. Subjects will be considered exhausted if they answered “2 = occasionally or a moderate amount of time (3–4 days)” or “3 = most or all the time (5–7 days)”
Weakness	Evaluated using handgrip strength with same procedure and cutoff points as sarcopenia assessment.
Slowness	Assessing the usual walking speed of subject along a distance of 4.6 m. Those with >7 seconds (for male with height <173cm or female with height < 159 cm) and >6 seconds (for male with height >173 cm or female with height >159 cm), will be categorized as positive for frailty in this category
Low physical activity	Based on RAPA questionnaire. Subjects who are classified as sedentary or underactive will be considered as positive for frailty in this category (Mohd Hamidin *et al.* 2018).

CES-D: Center for Epidemiologic Studies Depression; RAPA: Rapid Assessment of Physical Activity

be used to personally identify the participants; instead, they will be displayed in groups. The specific subjects' identities won't be disclosed following the publication. All study-related data will be kept safe and accessible to a limited number of people. Study records denoted by respondent codes will be kept apart from all other records that include names or other personally identifiable information, such as registration forms and informed consent forms.

The corresponding author can provide the datasets used and/or analyzed for this study upon reasonable request.

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DECLARATION OF CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

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Research Article

The Effect of Sacha Inchi Tempe on Blood Glucose, HOMA-IR, and TNF-α in Rats with Metabolic Syndrome

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ABSTRACT

This research aimed to evaluate the impact of sacha inchi tempe (*Plukenetia volubilis* L.) on Fasting Blood Glucose (FBG), Homeostatic Model Assessment for Insulin Resistance (HOMA-IR), and Tumor Necrosis Factor Alpha (TNF-α) levels. In addition, metabolic syndrome was induced in 36 male Wistar rats aged 2 months at 150–200 g weight by giving a High-Fat High-Fructose Diet (HFFD) for 2 weeks. The extract was administered through oral gavage in dose-dependent manner and rats were allocated into 6 groups, namely: 1). Normal control or K0; 2). Negative control or K-; 3). Positive control or K+ with 0.18 mg/200 g BB of simvastatin; 4). Intervention with 0.9 g sacha inchi tempe or P1; 5). Intervention with 1.8 g sacha inchi tempe or P2, and; 6). Intervention with 3.6 g sacha inchi tempe or P3. Meanwhile, normal chow rats were used and served as the control group. After 2 and 5 weeks of induction and intervention, blood was drawn to determine FBG. Blood insulin was examined after 5 week of intervention. Rats were euthanized at the end of the intervention for hepatic TNF-α analysis before calculating HOMA-IR. The result showed that there was a significant difference ($p<0.05$) in FBG, HOMA-IR and hepatic TNF-α levels after sacha inchi tempe treatment. Rats receiving the highest dose of sacha inchi tempe had the most significant reduction ($p<0.05$) in FBG, HOMA-IR and hepatic TNF-α, when compared to simvastatin group. Therefore, sacha inchi tempe could attenuate glycemic and inflammation profiles in metabolic syndrome.

INTRODUCTION

Metabolic syndrome is a group of abnormalities, including insulin resistance, hypercholesterolemia, hypertriglyceridemia, hypertension, central obesity, and low High-Density Lipoprotein Cholesterol (HDL-c). In this context, the risk of cardiovascular disease and type 2 diabetes mellitus can be increased twofold and fivefold, respectively (Wang *et al.* 2020). The prevalence of metabolic syndrome is increasing worldwide and has reached 21.66% in Indonesia (Herningtyas & Ng 2019).

The pathophysiology of metabolic syndrome starts with genetic factors, increased calorie intake containing simple sugar and high fat, and low physical activity. This causes the accumulation of visceral fat which increases Reactive Oxygen Species (ROS) and Renal Artery Stenosis (RAS), hence triggering an increase in angiotensin II. Similarly, inflammatory factors such as IL-6, TNF-α, CRP, and fibrinogen, are increased. Free Fatty Acids (FFA), lipogenesis, gluconeogenesis, and triglycerides are increased while insulin and glucose uptake are decreased. Hyperglycemia conditions also increase lipid production which is accumulated in the liver to enhance insulin resistance (Rojanaverawong *et al.* 2023; Wang *et al.* 2016).

Sacha Inchi is a tropical plant rich in oil (35%–60%), protein (25%–30%), minerals, essential amino acids, and vitamin E (Torres-Sánchez *et al.* 2023). The major fatty acid compounds in this plant are linolenic and linoleic acids. The α-Linolenic Acid (ALA) is an essential fatty acid found in the ω-3 group. ALA serves as a substrate for the production of Docosapentaenoic Acid (DHA), Eicosapentaenoic Acid (EPA), and other longer-chain of unsaturated ω-3 fatty acids (Baker *et al.* 2016). The compound has antioxidant activity by increasing the GSH/GSSG ratio and GSH content without modification of the levels in the liver (Rincón-Cervera *et al.* 2016). Meanwhile, sacha inchi has the highest ALA compared to other tropical nuts such as peanuts, cashews, tropical almonds, and Brazil nuts. The content is 10–100 times higher than other nuts, hence foods made from the plant contain high levels of ALA (Cardoso *et al.* 2017; Ng *et al.* 2015; Rico *et al.* 2016; USDA 2021).

Tempe is a traditional Indonesian food prepared by fermenting soybeans using *Rhizopus spp.* The fermentation process causes tempe to have several advantages such as a reduction in anti-nutritional substances, elevation in vitamins and high bioavailability of carbohydrates, proteins, and fats (Ahnan-Winarno *et al.* 2021; Bueno-Borges *et al.* 2018; Nurrahman *et al.* 2013). The food contains genistein, daidzein, and β-sitosterol, which influence blood glucose balance and prevent cancer, heart disease, and type 2 diabetes (Huang *et al.* 2018). Previously, Ulfa *et al.* (2022) made tempe from kedawung seeds which improved hemoglobin and albumin in protein-energy deficient rats. In addition, sorghum tempe was reported to reduce Low-Density Lipoprotein-cholesterol (LDL-c) and malondialdehyde levels in rats (Khoirun Nisa *et al.* 2021). Sacha inchi tempe contains 1.97% ash, 28.51% moisture, 10.06% carbohydrates, 38.43% fat, 20.50% protein, 1.60% Saturated Fatty Acids (SFA), 2.68% Monounsaturated Fatty Acids (MUFA), 32.99% Polyunsaturated Fatty Acids (PUFA), 14.18% Linoleic Acid (LA), and 18.82% α-Linolenic Acid (ALA). The best duration of the fermentation is 72 hours but there is limited research on sacha inchi tempe (Salam *et al.* 2023). Moreover, sacha inchi is used as an additional ingredient in making yogurt. Partial substitution of cow's milk with the seeds significantly increases ALA in yogurt (Vanegas-Azuero & Gutiérrez 2018). Tempe and other fermented soybean products, such as natto, miso, kinema, doenjang, douchi, and chungkookjang, can improve metabolic syndrome (do Prado *et al.* 2022).

Sacha inchi tempe has the potential against metabolic syndrome due to the bioactive ingredients. Therefore, this research aims to determine the effect of giving sacha inchi tempe on Fasting Blood Glucose (FBG), insulin resistance (HOMA-IR), and pro-inflammatory factor TNF-α in male Wistar rats with metabolic syndrome.

METHODS

Design, location, and time

This experimental research was carried out with a randomized post-test control group design. Sacha Inchi seeds were obtained from Kuningan, West Java, Indonesia and the research on experimental animals was carried out in a facility at the Center for Food and Nutrition Studies, Gadjah Mada University, D.I. Yogyakarta from January to March 2024. The ethical clearance was obtained by The Research Ethics Committee of Faculty Medicine, Diponegoro University No. 003/ EC-H/ KEPK/FK-UNDIP/I/2024.

Materials and tools

Sacha inchi tempe as treatment was made by researchers from sacha inchi seeds and "Raprima" brand mold. Induction of metabolic syndrome was done by administering a High-Fat and Fructose Diet (HFFD) for 2 weeks. The diet given was 15 g–20 g/day (10% of body weight), consisting of pork fat (20%), cholesterol (1.5%), cholic acid (0.5%) and fructose as much as 1 mL/200 g per body weight of rats (65% fat, 25% carbohydrate, and 10% protein). Lee's index was measured using a Medline and a digital scale. Triglycerides were measured by Triglycerides GPO FS Kit (DiaSys, Germany). HDL-c was measured by Cholesterol CHOD FS Kit (Diasys, Germany). The German-made S-2 sphygmomanometer was used to measure blood pressure using the tail-cuff method. FBG was measured by Glucose GOD FS Kit (DiaSys, Germany). Insulin levels were measured by insulin ELISA Kit (FineTest, China). TNF-α levels were measured by TNF-α ELISA Kit (FineTest, China).

The tools used in this research were cages, food dish, drinker, Eppendorf, 0.5 ml EDTA tube, oral gavage, syringe, microhematocrit, water bath, centrifuge, cooling bath, microplate reader, Uv-Vis spectrophotometer, and cuvette.

Procedure

Sacha inchi tempe. Sacha inchi seeds were washed thoroughly and soaked for 2 hours. After soaking, sacha inchi seeds were boiled for 30 minutes and then resoaked for 24 hours until mucus appeared on the surface of the water. Subsequently, the seeds were drained and cleaned with the attached epidermis. Sacha inchi seeds were steamed for 15 minutes, spread evenly in a container, cooled, dried, and sprinkled with 0.5% tempe yeast of the total weight. The seeds were packaged using banana leaves and stored in a dark, damp place for 72 hours (Salam *et al.* 2023).

Animal research. According to the result obtained, thirty-six male Wistar rats weighing178.31±3.40 g were used. In this context, the preadult and adult ages were represented by 2-month-old rats (Fitria *et al.* 2018) and the acclimatization process was carried out for seven days in 12 light-dark cycles with a temperature control of 25±2°C. Additionally, diet of 15 g−20 g CP594 feed were administered with water and randomly assigned to six groups. This research comprised normal control (K0), metabolic syndrome (K-), simvastatin (K+), sacha inchi tempe dose I (P1), sacha inchi tempe dose II (P2), and sacha inchi tempe dose III (P3). Groups K-, K+, P1, P2, and P3 received 15 g/day of HFFD for 14 days in the process of inducing metabolic syndrome, while K0 was given standard diets and ad libitum water. The three components of metabolic syndrome included obesity (Lee Index >300), triglyceride levels >150 mg/dL, HDL levels <40 mg/dL, FBG levels >100 g/dL, and systolic blood pressure >130 mmHg (Srikanthan *et al.* 2016). After 14 days, group K- was given standard feed only, group K+ was given standard feed and simvastatin (0.18 mg/200 g BW/day), group P1, P2, and P3 received standard feed + 0.9, 1.8, and 3.6 g/200 g BW/day sacha inchi tempe, respectively. The extract was grinded before adding distilled water to obtain a 5 mL volume once a day for 5 weeks through oral gavage and the rats fasted for 8 hours before the blood was drawn to determine FBG and insulin levels. Meanwhile, the tissue for hepatic TNF-α levels was obtained by euthanizing the sample with the ketamine overdose method.

Blood was extracted twice through the orbital sinus using the retro-orbital plexus method after 14 days of HFFD and 5 weeks of tempe intervention. Meanwhile, the serum was obtained by leaving blood at room temperature and centrifuging for 15 minutes at 4,000 rpm. Liver tissue was taken at the end of the research to check TNF-α levels.

Metabolic profile analysis. GOD-PAP, Enzymatic Calorimetric Test of Glucose Oxidase Phenol 4-Aminophenazone were used to obtain FBG levels (Subiyono *et al.* 2016). Additionally, insulin levels were reported by reacting serum with monoclonal anti-rat insulin (antibodies) coated in microplate wells and the reagents in the ELISA Kit. The analytical process was carried out according to the kit's instructions (Shen *et al.* 2019) while Lee index calculation was obtained from the weight and length of rats. The weight was determined by weighing rats using a digital scale. Similarly, the length of the bodies was measured from the snout to the base of the tail using a Medline. Lee index was calculated using the following formula:

$$\text{Lee index} = \frac{\sqrt{body\ weight\ (g) x\ 10}}{body\ length\ (mm)}$$

Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) measurement. HOMA-IR is used in blood test results to assess insulin resistance formulated as (fasting insulin × fasting glucose) / 405, with glucose and insulin in units of mg/dL and μIU/mL, respectively (Putri *et al.* 2022).

TNF-α levels measurement. TNF-α levels were measured using liver tissue and a total of 0.1 g was mixed with 1 mL of PBS pH 7.2 then crushed using a mortar and centrifuged at 5,000 rpm for 5 minutes. The supernatant was transferred to a microtube and the measurement was carried out using the sandwich ELISA technique in line with the protocol specified for the kit (Sholihah *et al.* 2018).

Data analysis

The mean and standard deviation of all the collected data were reported using SPSS (IBM, version 22). Statistical tests were carried out to determine the differences before and after the intervention in FBG. In addition, the paired t-test was adopted for the FBG before and after intervention. One-way ANOVA statistical test, followed by posthoc Tukey HSD with a significant value of $p<0.05$ was carried out to determine the difference of FBG, HOMA-IR, and TNF-α among groups.

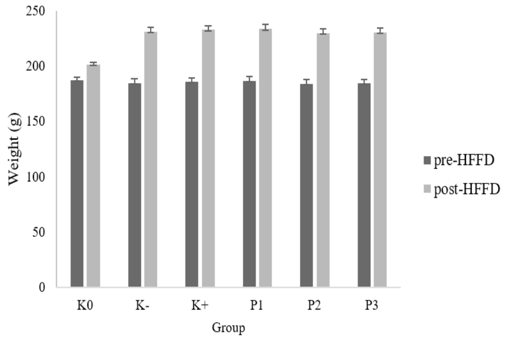
RESULTS AND DISCUSSION

A total of 36 rats were divided into six groups since no death was recorded during the intervention. The results showed that sacha inchi tempe significantly lowered FBG levels, insulin resistance as measured by HOMA-IR index, and TNF levels seen in Figure 2. Sacha inchi tempe was able to increase insulin levels in metabolic syndrome rats.

According to Salam *et al.* (2023), the optimum fermentation time was 72 hours and sacha inchi Tempe contained 18.82% ALA. In this research, 0.9 g, 1.8 g, and 3.6 g of sacha inchi tempe were equivalent to 169.38 mg ALA, 338.76 mg ALA, and 677.52 mg ALA, respectively.

Body weight. Group K0 was not given HFFD induction while the other 5 groups received the induction. There were changes in the body weight and the greatest increase in the HFFD-induced group was the K+ group. K0 showed the smallest increase in body weight because the group was not induced by HFFD. The weight gain in K0 was lower when compared to the control in Battung *et al.* (2019) with a weight gain of 22.6 g due to the age difference. In addition, the standard deviation value was relatively large compared to the results. Groups K-, K+, P1, P2, and P3 showed a higher increase in body weight compared to K0 ranging from 46–47 g in each group given HFFD. The results showed a greater increase in body weight compared to research using a hypercholesterol diet and 60% fructose, which gave an increase of about 30 g in 2 weeks (Barrios-Ramos 2014). Hussain *et al.* (2019) stated that weight gain was increased by 3.4g/day and 8g/day in the control and HFFD, respectively. On the 14th day, the weight change of the control and HFFD groups were 47.6 g and 112 g, respectively. A statistical test with one-way ANOVA showed no significant difference in the pre-HFFD group, hence the sample had been randomized successfully. However, these results showed the success of HFFD induction on body weight, as reported in Figure 1.

Metabolic syndrome biomarkers. After HFFD administration for 2 weeks, rats in the K-,



K0: Healthy + standard diet; K-: Metabolic syndrome + standard diet; K+: Metabolic syndrome + 0.18 mg/200 g b.w./day of simvastatin; P1: Metabolic syndrome + 0.9 g sacha inchi tempe; P2: Metabolic syndrome + 1.8 g sacha inchi tempe; P3: Metabolic syndrome + 3.6 g sacha inchi tempe

Figure 1. The mean weight of rats before and after High-Fat High-Fructose Diet (HFFD) induction

K+, P1, P2, and P3 groups experienced an increase in Lee Index, HDL-c, FBG, and systolic blood pressure, as well as a decrease in HDL-c. This research showed a higher Lee index in the HFFD group compared to El-Saka who used 3–4 weeks-old rats (El-Saka *et al.* 2023). Based on Zhang in Sprague Dawley rats fed HFFD for 20 weeks, the HDL-c value was 48 mg/dL (Zhang *et al.* 2022). However, the results are in line with Hidayati who experienced a significant decrease in HDL-c after administration of HFFD (Hidayati *et al.* 2020). Hypertriacylglycerolemic rats fed HFFD for 5 weeks showed a systolic blood pressure of 133 mmHg, lower than the results. However, rats experienced hypertension (Sasváriová *et al.* 2019) and higher FBG was reported after HFFD administration. Previous research showed a normal HFFD of 84 mg/dL after 2 weeks. Hidayati who gave HFFD for 4 weeks reported a significant increase in FBG (Hidayati *et al.* 2020) but the level of triglycerides did not improve after the administration. Based on Barrios-Ramos triglycerides increased when HFFD was given for 7 weeks. Even though HFFD was given for 2 weeks, TG was 57 mg/dL, lower than any group (Barrios-Ramos 2014). However, rats had fulfilled >3 of the 5 metabolic syndrome criteria and the condition was achieved (Srikanthan *et al.* 2016). The differences were due to the type and age of rats, as well as the duration of HFFD, as presentend in Table 1.

Table 1. The mean biomarker of Lee index, TG, HDL-c, FBG, and blood pressure after HFFD induction

Biomarker	Normal value	Groups of treatment (Mean±SD)					
		K0	K-	K+	P1	P2	P3
Lee index	<300	284.11±3.01	335.99±5.81	337.46±6.08	339.85±4.28	336.55±2.98	338.31±6.66
TG (mg/dL)	<150	70.91±2.48	136.04±2.56	135.57±2.34	134.16±1.97	133.69±3.11	132.16±3.31
HDL-c (mg/dL)	>40	82.65±1.27	23.58±2.38	23.81±2.15	24.72±1.47	23.47±1.27	23.24±1.69
FBG (mg/dL)	70–100	71.73±2.87	148.65±2.41	149.26±2.22	151.22±1.75	150.34±2.63	148.79±1.37
Systolic blood pressure (mmHg)	<130	96.00±3.41	139.00±2.10	138.83±2.48	137.33±2.07	137.00±2.68	137.17±3.19

K0: Healthy + standard diet; K-: Metabolic syndrome + standard diet; K+: Metabolic syndrome + 0.18 mg/200 g b.w./day of simvastatin; P1: Metabolic syndrome + 0.9 g sacha inchi tempe; P2: Metabolic syndrome + 1.8 g sacha inchi tempe; P3: Metabolic syndrome + 3.6 g sacha inchi tempe; TG: Triglyceride; HDL: High-Density Lipoprotein; FBG: Fasting Blood Glucose; HFFD: High-Fat High-Fructose Diet; SD: Standard Deviation

Caloric restriction. The K-, K+, P1, P2, and P3 groups were given a normal diet during the 5 weeks of the intervention period. Apart from the intervention received in the form of simvastatin or tempe, these rats were given caloric restriction because HFFD was stopped.

FBG levels. The administration of HFFD for 14 days increases FBG. Exposure to fructose indirectly creates compensatory hyperinsulinemia. This condition includes fructose transporter (GLUT5), which contributes to insulin resistance and raised plasma glucose concentrations (Barrios-Ramos 2014). Since fructose has lipogenic (fat-producing) properties, a considerable amount enters the liver and accumulates as triglycerides and cholesterol. This reduces insulin sensitivity and increases the resistance and glucose intolerance. A high-fat diet contributes to increasing triglycerides and causes insulin resistance (Wong *et al.* 2016). In this research, triglycerides were not higher than 150 mg/dL, hence fructose played a greater role in increasing FBG.

There was a significant difference in each group before and after 5 weeks of intervention ($p<0.001$), as reported in Figure 2A. In addition, the level of FBG was increased in the K+, P1, P2, and P3 groups. This shows that the K+ group as well as P1, P2, and P3 reduce FBG values. From the post hoc test, the difference in FBG value (ΔFBG) between K0 and K- is not significant. This is because the two groups were not given simvastatin or sacha inchi tempe. In the K- group and those given the simvastatin/sacha inchi tempe intervention, the FBG value increased and decreased, respectively. This showed the effect of the intervention on reducing FBG levels. Groups P1 and P2 were not significant, while P3 was significant from P1 and P2. In addition, groups P1, P2, and P3 were not significantly different compared to the simvastatin group (K+).

FBG levels in metabolic syndrome rats group increased compared to K0. Metabolic syndrome could trigger insulin resistance, inflammation, and oxidative stress to increase FBG levels (Fahed *et al.* 2022). Meanwhile, a tight range of glucose is maintained to ensure a steady supply. This is because the liver is the primary organ responsible for controlling blood glucose, insulin, glucagon, and epinephrine (König *et al.* 2012).

Simvastatin group (K+) reduced FBG significantly compared to those without intervention (K-). In addition, simvastatin is a member of statin, an HMG-CoA reductase inhibitor used in the treatment of metabolic syndrome (Chan & Watt 2011). This FBG-decreasing effect can be caused by simvastatin in reducing MDA and increasing GSH (Crespo & Quidgley 2015; Hadi *et al.* 2015). The antioxidant effect plays a role in decreasing HOMA-IR values. The results are in contrast to Fawzy Fahim where the administration of streptozotocin-induced diabetic rats did not significantly reduce FBG (Fawzy Fahim *et al.* 2019).

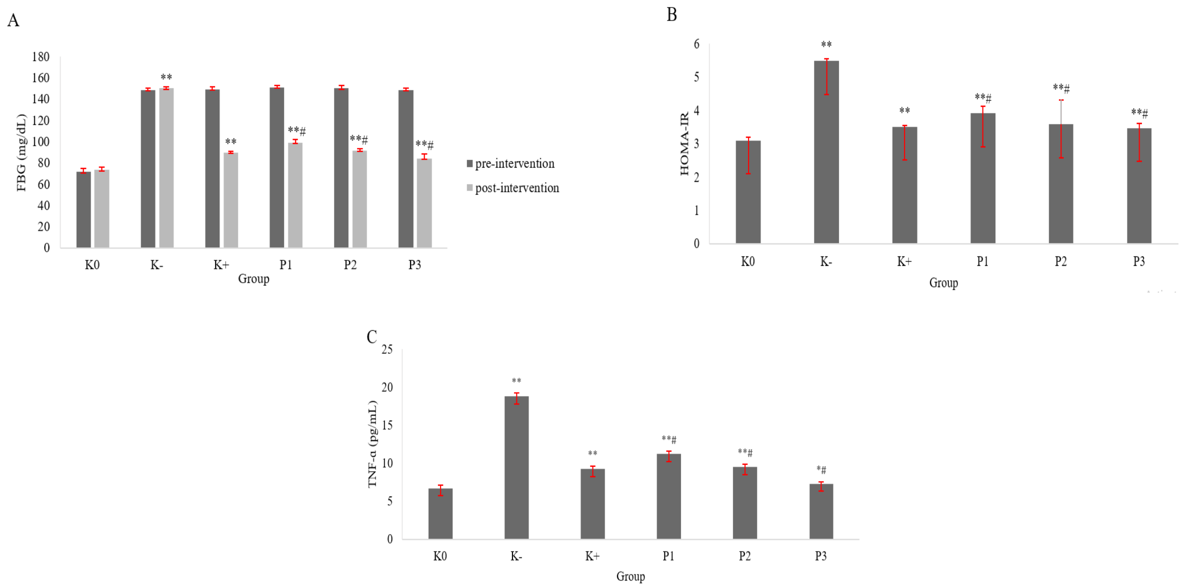
P1, P2, and P3 groups decreased FBG levels significantly when compared to K-. Sacha inchi tempe is rich in ALA and flavonoids used to suppress enzymes in gluconeogenesis such as Phosphoenol Pyruvate Carboxykinase (PEPCK) and G-6-Pase. This is the first research to discuss the effect of sacha inchi tempe on FBG.

Rojanaverawong reported that 1 mL/kg of the extract reduced FBG from 333 mg/dL to 225 mg/dL in 5 weeks (Rojanaverawong *et al.* 2023).

HOMA-IR. HOMA-IR was used to assess insulin resistance, by calculating the concentration and fasting plasma glucose in the context of diabetes mellitus (Putri *et al.* 2022). The value after sacha inchi intervention is shown in Figure 2B and there was a significant difference in the mean between groups ($p<0.001$). Meanwhile, insulin resistance and HOMA-IR values were affected by discontinuation of HFFD administration in rats receiving intervention (Barrios-Ramos 2014). HOMA-IR values of the intervention group P1, P2, and P3 were significantly different from K-. In this context, insulin resistance was reduced by the improvements in HOMA-IR values and sacha inchi tempe. Simvastatin group and P2 were not significantly different since P3 was better in reducing HOMA-IR value. Therefore, 3.6 g sacha inchi tempe had a better effect than simvastatin on HOMA-IR value in metabolic syndrome conditions. In the intervention group, P3 had the lowest HOMA-IR value, followed by P2 and P1. There were significant differences between groups P1, P2, and P3, hence the effect was dose-dependent. As the best dose, P3 reduced HOMA-IR value by 38.3% compared to the negative control. The results were in line with Rojanaverawong who showed a decrease in rats given sacha inchi oil. In this context, 1 mL/kg of sacha inchi oil reduced HOMA-IR value to 9 (Rojanaverawong *et al.* 2023).

A high HOMA-IR index signifies a disturbance in the body cells' absorption. The cafeteria diet commonly consumed tends to be high in sugar and fat, increasing visceral fat and triggering inflammation through increased TNF-α, IL-6, and decreased IL-10. In addition, there was an increase in FFA which causes oxidative stress. Insulin resistance may result from disruptions to signalling caused by oxidative damage and inflammation (Ahmed *et al.* 2022).

The decrease in HOMA-IR values after sacha inchi tempe intervention showed a repair response of target cells to activate the use of glucose. Sacha inchi tempe contained ALA, flavonoids, and β-sitosterol which could improve insulin sensitivity through the IRS-1/PI3K/Akt signaling pathway. This is achieved by increasing IRS-1 and p-Akt (Ser 473) protein expression in the liver (Rojanaverawong *et al.* 2023). The insoluble dietary fiber triggered the formation of acetate which played a role in improving insulin



Data are expressed as mean±standard error of the mean (n=6)

$^{*}p<0.05$; $^{**}p<0.001$ vs. the control group; $^{\#}p<0.001$ vs. the diabetic group, as analyzed with ANOVA followed by Tukey's HSD test

P: Treatment; K+: Simvastatin; K-: Diabetic group; K0: Normal control

Figure 2. The effect of sacha inchi tempe on (A) fasting blood glucose, (B) homeostatic model assessment for insulin resistance, and (C) tumor necrosis factor-alpha

sensitivity (Fu *et al.* 2022; González Hernández *et al.* 2019). Sacha inchi reduced triglycerides and triggered the formation of Very Low Density Lipoprotein (VLDL). In diabetic rats, sacha inchi oil decreased blood and pancreatic MDA levels as well as atrophic pancreatic islets (Wongmanee *et al.* 2024).

Hepatic TNF-a levels. Hepatic TNF-α levels in Figure 2C showed a significant difference in values between the treatment groups (P1, P2, and P3) and DM ($p<0.001$). Tempe treatment groups P1, P2, and P3 were significantly different from the K- group. This showed that administration of sacha inchi tempe reduced hepatic TNF-α levels. Meanwhile, P2 group was not significantly different from K+ which received simvastatin intervention in reducing hepatic TNF-α. P3 was better at reducing TNF- α levels compared to K+. In this context, 3.6 g sacha inchi tempe had a better effect than simvastatin on TNF-α levels in metabolic syndrome conditions. Conversely, P3 had the smallest levels of TNF-α, followed by P2 and P1. The group had an amazing effect on reducing hepatic TNF-α which caused a reduction of 61% compared to the negative control, while K+, P2, and P1 were reduced by 50.7%, 49.2%, and 40.1%, respectively. Groups P1, P2, and P3 had significant differences since sacha inchi tempe reduced TNF-α in a dose-dependent manner.

TNF-α is a cytokine produced in adipose tissue by macrophages. The production is proportional to adipose tissue mass and is related to insulin resistance. In addition, insulin resistance is influenced by the following mechanisms: 1). Inhibiting GLUT4 expression; 2). Stimulating lipolysis and increasing FFA levels; 3). Inhibiting insulin signaling through serine phosphorylation of IRS-1; 4). Inhibiting the synthesis of Peroxisome Proliferator-Activator Receptor γ (PPARγ) (Moller 2000).

The group receiving simvastatin experienced a decrease in TNF-α levels in line with another research that gave simvastatin 20 mg/day to peritoneal dialysis patients. The decrease in TNF-α was caused by the reduction in LDL-c levels. Meanwhile, the decrease in LDL-c caused a reduction in TNF-α production by endothelial cells, macrophages, and smooth muscle cells (Tugrul Sezer *et al.* 2007). Sacha inchi oil (1 mL/kg) reduces TNF-α by 2.9 pg/mL, which is lower than the result (Rojanaverawong *et al.* 2023).

Sacha inchi as the main component in tempe contains active ingredients such as ALA, α-tocopherol, and flavonoids showing anti-inflammatory properties in diabetic rats (Ghadge *et al.* 2016; Jamalan *et al.* 2015; Othman *et al.* 2021). In a research carried out by Rojanaverawong *et al.* (2023), the administration of sacha inchi significantly improved inflammatory cytokines and liver inflammation while maintaining the function and increasing insulin sensitivity (Ambulay *et al.* 2020). Moreover, oxidative stress and TNF-α expression resulted from ROS-mediated activation of p38 mitogen-activated protein kinase and NF-κB, triggering the release of extra TNF-α (Grandl & Wolfrum 2018). The limitation of this study is sacha inchi tempe was not compared with the seeds so that the effect of fermentation on the extract could not be determined.

CONCLUSION

In conclusion, a significant effect was shown by sacha inchi tempe in reducing FBG, HOMA-IR, and TNF-α levels. The best dosage for improving metabolic health of rats was a dose of 3.6 g. Since liver and pancreas were closely related to the glycemic profile, further research should be conducted to examine the histology of the organs.

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DECLARATION OF CONFLICT OF INTERESTS

The authors have no conflict of interest.

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Research Article

Anti-Hypertensive and Anti-Hypercholesterolemic Effects of Protein Hydrolysates from (*Phaseolus vulgaris)* L. in Functional Beverage

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ABSTRACT

The study aimed to formulate a functional beverage from common bean seeds, isolate the proteins and evaluate their anti-hypertensive and anti-cholesterolemic properties. White common beans (*Phaseolus vulgaris* L.) mature seeds were used to prepare the beverage. Proteins isolated from the beverage were subjected to digestion with pepsin and combined enzymes including trypsin, thermolysin, and chymotrypsin. The unhydrolyzed beverage and protein hydrolysates were subsequently tested for Angiotensin Converting Enzyme (ACE) inhibition and cholesterol micellar solubility inhibition. The results showed that both unhydrolyzed and hydrolyzed proteins exhibited blood pressure and cholesterol-lowering properties, with high ACE inhibition (77.60%) and cholesterol micellar solubility inhibition (27.38%). The formulated functional beverage from white common bean seeds has potential for preventing hypertension and hypercholesterolemia. This study offers a theoretical foundation for the formulation of functional beverages or bean-based food products by food companies.

INTRODUCTION

In recent years, studies on functional foods from plant sources have increased dramatically because of their nutritional attributes and beneficial effects on health. Several studies focus on various food-derived proteins as sources of bioactive peptides that exhibit potent pharmacological properties and provide safety profiles to consumers (Antony & Vijayan 2021). Bioactive peptides have multifunctional properties that include immunomodulatory, antibacterial, antihypertensive, anticancer, antioxidative, antilipidic, anti-inflammatory, hypocholesterolemic, and the property of minerals binding (Angeles *et al.* 2021; Manzoor *et al.* 2022). Bound peptides must be released from their protein sources through enzymatic cleavage or fermentation to produce bioactivity (Tadesse & Emire 2020). Digestive enzymes such as trypsin, pepsin, or chymotrypsin produce peptides with varied chain lengths which may directly affect transportation to different tissues in order to elicit a favorable impact on the organism (Jakubczyk *et al.* 2017).

One of the most cultivated legumes in the Philippines particularly in Cordillera Administrative Region (CAR) is common beans. These sources of nutrients and bioactive compounds provide physiological advantages, reducing the susceptibility of developing type 2 diabetes and cardiovascular diseases, preventing various cancers, and managing some metabolic processes (Sparvoli *et al.* 2021). Chronic conditions significantly increase the expense of long-term medical treatment and are among the major causes of death worldwide (Chen *et al.* 2019). Seeds of common beans are rich sources of proteins, a class of macromolecule that serve an important role in the food industry as components which provide nutritional, functional, and sensory qualities to foods (Aryee *et al.* 2018). Bioactive peptides generated from plant proteins can be used for creating innovative functional foods, presenting a feasible alternative to peptides from proteins of animal sources (Lopes *et al.* 2023). Plant-based proteins have been recommended for their nontoxic characteristic, affordability, wide availability, and various biological functions (Fan *et al.* 2022).

Plant-based beverages have gained popularity as an alternative to traditional drinks due to several reasons, such as their health-promoting properties, bioavailability of active ingredients, sensory characteristics, and suitability for consumers with lactose intolerance and dairy milk allergies (Arbach *et al.* 2021; Aydar *et al.* 2023). Legumes are viable solution to a range of food industry challenges since they offer a promising protein source in plant-based diet (Aydar *et al.* 2023). There are various legume and cereal-based beverages in the market. However, for legumes, soybean milk is still dominant and studies on common bean beverages are scarce.

Bean-based beverages offer nutraceutical benefits and regular consumption by consumers should be encouraged. These could introduce a prospective market for health products from a low-cost sustainable source. At present, information on common bean beverage formulations and their nutraceutical properties is limited. The outcome of the current study will serve as a theoretical basis in-vitro for the investigation of innovative food development.

This research sought to formulate a functional beverage from common bean seeds and assess its potential for lowering blood pressure and cholesterol. Specifically, it isolated the protein from the formulated functional beverage, digested the protein, and evaluated the blood pressure and cholesterol-lowering activities of the hydrolyzed and unhydrolyzed proteins isolated from the formulated beverage.

METHODS

Design, location, and time

The in-vitro study was carried out at the laboratory of the Institute of Chemistry, University of the Philippines Los Baños, Philippines from January to February 2020.

Materials and tools

The common bean seed samples were obtained from local markets in Benguet, Philippines. For the identification process, the complete parts of the fresh plant samples, including the leaves, seeds and pods, were brought to the Museum of Natural History in the University of the Philippines Los Baños where they were identified as *Phaseolus vulgaris* L. Analytical grade chemicals used in the study were purchased from local distributors.

Procedures

Formulation of functional beverage. White common beans (*Phaseolus vulgaris* L.) mature seeds were the main ingredient in the preparation of functional beverage. The blending method with a few modifications was adopted from Afroz *et al.* (2016). About 100 g of dried mature seeds were soaked separately for four hours with 1 liter of warm water containing a small amount of sodium bicarbonate. Sodium carbonate was added to eliminate the bitterness and anti-nutritional factors. After manually removing the seed coats, the seeds were rinsed with distilled water. Dehulled seeds were then placed in a stone mill with 1 liter of hot water poured in. The seeds were ground and then filtered using a filter machine with cheesecloth. The filtrate was boiled over low/medium heat for ten minutes with constant stirring. After adding 45 g of sugar, the mixture was boiled for 2 more minutes. The prepared beverage was cooled to 70^{o}C before mixing one teaspoon of vanilla and was then set aside to cool to approximately 25^{o}C before being stored in a refrigerator set at 2^{o}C .

Isolation of protein from common bean beverage. Precipitation of proteins was conducted following the methodology developed by Hermanto *et al.* (2019), with some modifications. With stirring, 1.0 N NaOH was added to about 450 mL of the beverage to achieve a pH of 8.5. The solution was centrifuged at 5,600 x g for 20 minutes at 4^{o}C . The supernatant layer was decanted and 1.0 NHCl was stirred in to achieve a pH of 4.5. It was further centrifuged for another 20 minutes at 5,600 x g at 4^{o}C . The precipitate was collected and kept in a freezer.

Digestion of isolated protein. In order to imitate the digestion of proteins in human, enzymes pepsin, trypsin and chymotrypsin were employed. Pepsin, an aspartic protease in the stomach functions at optimum pH range between 1 and 2. Subsequently, during the digestion with pancreatic enzymes, such as trypsin, peptide bonds are cleaved after basic amino acids while chymotrypsin acts after aromatic amino acids (Fu *et al.* 2021). Peptides produced by thermolysin exhibited a high antihypertensive potential of peptides (Garcia *et al.* 2016) and was then used to enhance the production of bioactive peptide. The isolated protein sample was digested with pepsin using a 20:1 (v/v) substrate-to-enzyme ratio at pH 2.0 for one hour, and combined with enzymes (trypsin, thermolysin, and chymotrypsin) to simulate the digestion that occurs in the digestive system except for thermolysin. Then, the mixture was placed in a water bath (Benchmark Scientific SB-12L Shaking Water Bath) at a temperature of 25°C. The digestion process was terminated by immersing the mixture in a boiling water bath for 5 minutes. Then, the mixture was promptly stored in a freezer at -2°C. Prior to sample utilization, the mixtures were separated in a centrifuge at 4°C for 2 minutes at 12,857 x g. The supernatant was collected and loaded onto a gel for subsequent electrophoresis.

Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE). Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) was performed according to the method of Laemmli (1970), with slight modifications, using an 8% stacking gel and a 10% resolving gel. The solutions were prepared by diluting with the extraction buffer to obtain the desired concentration. A buffer of 1:1 (v/v) sample to 2x Laemmli was prepared and placed in Eppendorf tubes. The solutions were then mixed using a vortex mixer and heated in a boiling water bath for 5 minutes. Then, the solutions were cooled to approximately 25°C.

About 20 µl of each sample was put into the prepared gel and allowed to migrate down the gel at 90 V using a Biorad Mini-PROTEAN® Tetra cell until the dye front was approximately 0.5 cm from the bottom of the gel. The resolving gel was carefully removed from the plates and placed in a container for staining overnight using Coomassie Brilliant Blue R-250. A solution consisting of 50% methanol and 10% glacial acetic acid was used to destain the gel.

Extraction of Angiotensin-Converting Enzyme (ACE) from pig's lungs. The extraction process was conducted according to the method outlined by Dumandan *et al.* (2014) with modification on solvent and buffer used. The ACE was extracted in the laboratory due to its cost-efficiency. Previous research has shown that it provides reliable results. Initially, the cleaned and minced fresh pig's lungs were suspended in acetone and then homogenized using blender. The resulting mixture was filtered and air dried to evaporate the solvent residue that could interfere with further extraction steps. Subsequently, the dried residue was defatted using hexane to remove the lipids or fats that could contaminate the protein. Thus, a more purified extract was produced. The sample was then air dried to eliminate the remaining solvent that could impede the ACE extraction and its activity. To extract the Angiotensin Converting Enzyme (ACE) from the dried powder, 100 mM sodium borate buffer (pH 8.3) was added and then incubated at 4°C for 3 hours. Afterwards, it was centrifuged at 11,000 x g for 60 min at 4°C. The reddish supernatant obtained was the ACE extract, which was stored at -20°C.

ACE inhibitory activity assay. The assay for ACE inhibitory property was measured based on the method of Dumandan *et al.* (2014) with modifications on buffer and quantity of chemicals used. Assay mixtures for the sample, control, and blank were prepared in three replications. Captopril, an ACE inhibitor drug used in the management of high blood pressure, was used as the positive control. Each sample assay mixture contained 25 µL of 0.1 M sodium phosphate buffer, 25 µL of 0.3 M NaCl, 50 µL of 5 mM Hippuryl-L-Histidyl-Leucine (HHL), and 50 µL of protein hydrolysates. The mixtures were pre-incubated for 5 minutes at 37^{o}C. Meanwhile, the blank also contained similar amounts of solutions in the sample mixtures except that instead of protein hydrolysate, 0.1 M sodium phosphate buffer with volume of 50 µL was introduced. The control contained 25 µL of 0.1 M sodium phosphate buffer and 125 µL of 1 N HCl. To initiate the reaction, 25 µL of ACE extracted from pig's lungs was introduced to all mixtures, excluding the control. Subsequently, the mixtures were placed in shaker for 30 minutes at 37^{o}C. To stop the chemical reaction, about 150 µL of 1 N HCl was added to the sample and blank assay mixtures, while 25 µL of ACE was allowed for the control. Hippuric acid was obtained through extraction process involving vigorous shaking of the mixtures with 750 µl of ethyl acetate using a vortex for 15 seconds, followed by centrifugation for 2 minutes at 3,600 x g. The upper layer was separated and the solvent was allowed to evaporate in a steam bath. A total of 500 µL distilled water was used to dissolve the extracted hippuric acid and the resulting mixture was vigorously mixed using a vortex mixer. The sample absorbance was at 228 nm.

ACE inhibition activity was determined using the following equation:

$$\text{Inhibitory activity (\%)} = \left(\frac{\text{B-A}}{\text{B-C}}\right) \times 100\%$$

Where A=Absorbance in the presence of both ACE, HHL and inhibitor

B=Absorbance of blank with ACE and HHL but without inhibitor

C=Absorbance of control

The protein concentrations used in the assay were 1.00 mg/mL for crude and 0.25 mg/mL for partially purified fractions. The concentration of the extract that inhibited 50% of the ACE activity was the IC_{50} value, which was determined by measuring the ACE inhibitory activity and derived from the inhibition graph plotted for the concentration of protein hydrolysates.

Cholesterol micellar solubility inhibition. Cholesterol micelles were generated as a model for the micelles found in the human gut by sonicating 10 mM sodium taurocholate, 0.4 M cholesterol, 1 mM oleic acid, and 132 mM NaCl in 15 mM sodium phosphate (pH 7.4). Assessing the amount of cholesterol that remains within micelles when agents that destabilize or displace micelles are present, is a significant basis for determining the efficacy of protein or peptide hydrolysates in cholesterol reduction (Zhang *et al.* 2012).

The cholesterol micellar solubility inhibition was assessed based on the method described by Zhang *et al.* (2012), with modification. Micellar solution was prepared by sonication using Omni Sonic Ruptor 400. The solution contained 10 mM sodium taurocholate, 0.4 mM cholesterol, 1 mM oleic acid, 132 mM NaCl and 15 mM sodium phosphate (pH 7.4). To each 400 µL of the sample, positive control (cholestyramine) and blank, 450 µL of the micellar solution was added. The samples were then incubated at 37°C for 24 hours. The mixtures were centrifuged at 37°C for 30 minutes at 18,845 x g, and supernatant was collected.

One hundred µL of glacial acetic acid was mixed with 80 µL of the supernatant, followed by the addition of 120 µL of a color reagent. The mixtures were then incubated at 37°C for 15 minutes. Standard solutions with varying concentrations from 0 mg/mL to 0.06 mg/mL cholesterol in glacial acetic acid were prepared. The absorbance of each solution was read at 560 nm using a UV-vis spectrophotometer (Thermo Scientific Multiscan GO, Finland). The generated calibration curve was used to determine the amount of cholesterol that remained in the solution. The following equation was used in the calculation of the cholesterol micellar solubility inhibition (Zhang *et al.* 2012):

$$\% \text{ Cholesterol solubility inhibition} = [(Co - Cs)/Co]*100\ \%$$

Where: Co=Cholesterol concentration of original micelles

Cs=Cholesterol concentration of micelles with hydrolysates/positive control

Data analysis

All samples were analyzed in three replications. The analyses were repeated and experiments were conducted at least twice. ANOVA (one-way analysis of variance) was utilized to test the significance of differences ($p<0.05$) between conditions followed by Tukey's HSD test post hoc analysis. GraphPad Prism 6 was used for statistical analyses.

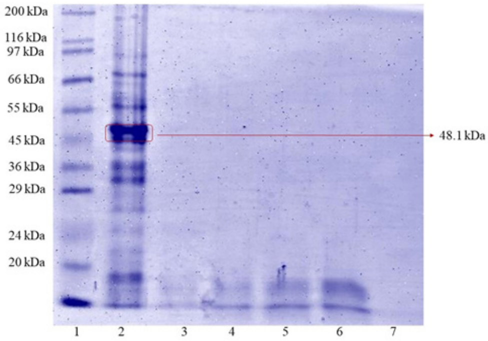
RESULTS AND DISCUSSION

Digestion of proteins

Cleavage specificity of proteolytic enzymes, degree of digestion, and stability of peptides during hydrolysis are crucial in the production of bioactive peptides (Mazorra-Manzano 2018). Liberated peptides may comprise 2 to 20 amino acids, and with small molecular weight, their assimilation capability into the small intestine is enhanced, increasing their ability to elicit physiological responses (Saad *et al.* 2021).

In the study, total protein isolated was digested with pepsin and then followed by a combination of enzymes: trypsin, thermolysin and chymotrypsin, sequentially. Pepsin is an endopeptidase that cleaves peptide linkages on the amino-terminal side of aromatic amino acids like tyrosine, phenylalanine, and tryptophan. Trypsin acts on the carboxyl end of basic amino acids arginine and lysine. In contrast, chymotrypsin acts on the carboxyl terminal of amino acids specifically leucine and aromatic amino acids in peptide chains (Mangussad *et al.* 2021). Thermolysin, a zinc-dependent protease, catalyzes the hydrolysis at the hydrophobic residues including leucine, isoleucine, valine, and phenylalanine (Nielsen *et al.* 2019). This in vitro analysis does not exactly mimic the digestion process of food that occurs in digestive tract of human because interactions with other components and other physiological factors were not included. Thus, the observed result may not demonstrate the inherent bioactive peptides' stability against proteases present in gastrointestinal tract (Chew *et al.* 2019).

Electrophoretic profile of formulated functional beverage protein. The electrophoretogram from SDS-PAGE analysis of the functional beverage from common bean is illustrated in Figure 1. Densitometric analysis of the unhydrolyzed protein showed multiple bands



(1) Protein profile of molecular weight marker; (2) Unhydrolyzed crude protein of functional beverage protein; (3) 1-hour peptic digest: (4) 1-hour combined enzymes digest; (5) 6-hour combined enzymes digest; (6) 12-hour combined enzymes digest, and; (7) 24-hour combined enzymes digest

Figure 1. SDS-PAGE of formulated functional beverage and hydrolysates from *Phaseolus vulgaris* L

visible in Lane 2, ranging from 26.8 kDa to 180.6 kDa. The most prominent fraction of protein had a molecular weight of 48.1 kDa, with the highest band volume at 11.4%. This protein band may correspond to a subunit of a phaseolin, a vicilin-like 7S globulin which consists of subunit polypeptides that have molecular weights ranging from 43–54 KDa (Saad *et al.* 2021) and have isoelectric points within pH range of 5.6 to 5.8. The 7S globulin is the most predominant protein in Phaseolus legumes seeds that accounts for the 40%–60 % total protein content (Sathe 2016).

The disappearance of bands corresponding to functional beverage protein and the presence of faint low molecular weight bands (17.1–24.7 kDa) after hydrolysis of functional beverage suggest that the proteins extracted were degraded (Lanes 3–7). After 24 hours of combined enzyme digestion, the bands completely disappeared indicating that peptides were liberated from their parent protein. According to data from Saad *et al.* (2021), phaseolin is resistant to enzymatic hydrolysis, unlike vicilin. In the case of vicilin, the bands completely disappear. Various factors, such as temperature, hydrolysis conditions, and enzymes, have an impact on protein binding and changes in molecular weight.

Previous research conducted by Tagliazucchi *et al.* (2015) on cooked 'Pinto' beans showed similar findings on the existence of faint protein bands and their absence after digestion with specific enzymes. Cooking of the beans is a thermal treatment that enhanced the degree of in comparison to uncooked beans, making them more susceptible to enzymatic hydrolysis.

ACE inhibitory property of functional beverage. The formulated functional beverage protein hydrolysates of the common bean beverage have exhibited ACE inhibition (Table 1). Significantly, the highest ACE inhibition (77.60%) was obtained at the optimum hydrolysis time of 6 hours, suggesting that the peptides demonstrating ACE inhibitory property were liberated consistently at 6-hour hydrolysis time although compared to the positive control, this was noticeably lower. The findings are consistent with those of Gao *et al.* (2019) who observed that hydrolysis time influences the ACE inhibitory activity of cottonseed protein digests. They observed that ACE inhibition increased up to the 6th hour and then decreased with extended time. This could be associated to the degradation of peptides and the loss of their bioactivity (Mao *et al.* 2007).

The findings revealed that unhydrolyzed protein beverage exhibited 55.68% ACE which is significantly lower compared to the peptic digest (73.65%) and to the combined enzymes digest ranging from 68.93% to 77.60 at different time intervals. This suggests that in the unhydrolyzed

Table 1. ACE inhibitory property of hydrolyzed and unhydrolyzed crude protein (1.0 mg/mL) of functional beverage formulated frommature seeds of *Phaseolus vulgaris* L.

Crude protein from functional beverage formulated from *Phaseolus vulgaris* L. and its hydrolysates	% ACE inhibition* (Mean)	SD
Functional beverage (unhydrolyzed)	55.68[e]	0.26
Peptic digest (1 hour)	73.65[c]	0.73
1-hour combined enzymes digest	68.93[d]	1.97
6-hour combined enzymes digest	77.60[b]	1.76
12-hour combined enzymes digest	66.82[d]	0.75
24-hour combined enzymes digest	58.89[e]	1.73
Captopril (positive control)	85.43[a]	0.20

*All data represent the means of three measurements; According to Tukey's HSD test, there are significant differences between letter superscripts at $p<0.05$, after conducting a one-way ANOVA with a 95% confidence interval; SD: Standard Deviation beverage, the peptides remain bound in the protein resulting in reduced efficacy. On the contrary, the utilization of two or more enzymes has been found to improve the effectiveness of hydrolysates as opposed to a single enzyme (Luna-Vital *et al.* 2015).

The finding of this study indicates that 1-hr peptic digest (73.65%) is higher than the result of Hermanto *et al.* (2019), who observed the ACE inhibitory activity of soymilk pepsin hydrolysates ranging from 24.2%–58.3% obtained over a period duration of 0 to 4 hours. However, they observed the highest ACE inhibition activity of 79.31% of soymilk pepsin hydrolysates after 48 hours, which is comparable to the highest value obtained in this study at 6-hour combined digest. Despite the variations in the source of peptides, enzymes, and hydrolysis period, this may imply that the peptides with maximum activity were generated.

Furthermore, the size of peptide components could potentially contribute to variability in the ACE inhibition of protein hydrolysates. This indicates that small-sized peptides present in the protein hydrolysates possess a more bioactive property than the larger peptide components found in unhydrolyzed protein. In a study on black soybean glutelin hydrolysates by Zhang *et al.* (2019), smaller peptides that have less than 3 kDa molecular weight demonstrated a greater ACE inhibitory activity than peptides exceeding 3 kDA in size. The synergistic effect of component peptides plays important role in enhancement of biological activities of peptides (Shao *et al.* 2023). Consequently, the peptides formed during hydrolysis exhibit interactions that result in increased ACE inhibitory activity. Differences in strength of ACE reduction property discussed in previous studies could also be ascribed to the peptide length and proportion of hydrophobic amino acids, peptides molecular weight, and the existence of branched-chain amino acids such as Leucine (L), Isoleucine (I) and Valine (V) (Xu *et al.* 2021; Zheng *et al.* 2020). Although the unhydrolyzed beverage and all hydrolysates did not surpass the ACE suppression of the positive control, captopril, an ACE inhibitor prescribed to control hypertension, this signifies as promising sources of antihypertensive peptides.

ACE Inhibitory IC_{50}. The IC_{50} of the processed bean drink hydrolysates is 0.20 mg/ml at 6-hour digestion using combined enzymes. This value for the formulated drink is lower than that of fresh milk (IC_{50}=1.18 mg/mL) observed by Chen *et al.* (2007), indicating a higher ACE inhibitory property of the formulated drink after a 6-hour sequential hydrolysis. This also has a higher value, suggesting lower ACE inhibitory activity in comparison to the value obtained in IC_{50} of processed soya milk with 0.26 µg/mL and 8.75 µg/mL regular soya milk (Tomatsu *et al.* 2013).

ACE is a constituent of Renin-Angiotensin-Aldosterone System (RAAS) and it regulates blood pressure and maintains balance of water and electrolytes in the body (Jakubczyk *et al.* 2017). Angiotensin I, a decapeptide derived from angiotensinogen, is converted to angiotensin II, a potent vasoconstrictor, through the action of ACE. In addition, ACE deactivates bradykinin, a substance with vasodilatory property that also influences aldosterone production. Thus, suppressing this enzyme contributes to the lowering of blood pressure.

Cholesterol micellar solubility inhibition of functional beverage. The micelle generated with sodium taurocholate performs similar mechanisms in the solubilization of cholesterol in human digestion. The cholesterol lowering property of protein could be evaluated by determining the amount of cholesterol that remains in the micelles in the presence of micelle-destabilising or micelle-displacing agent (Zhang *et al.* 2012).

Table 2 shows the cholesterol lowering properties of proteins from the formulated functional beverage. The obtained results reveal that hydrolyzed proteins including pepsin (24.31%) and coupled enzymes (ranging from 19.71%–27.38%) have significantly higher inhibitory effects than unhydrolyzed proteins (14.89%). This suggests that peptides with biological activities should be liberated from proteins' primary sequences to facilitate their effect. Regardless of the enzyme, hydrolysis greatly enhances functionality (Dent & Maleky 2023).

The 6-hour combined digest of the functional beverage exhibited the highest inhibitory activity (27.38%) for cholesterol micellar solubility; although this was noticeably lower than the control, cholestyramine (60.45%). This could be related to the peptides' low concentration released during digestion. Moreover, the inhibitory activities were lesser than the value reported by Marques *et al.* (2015)

Table 2. Cholesterol micellar inhibitory property of hydrolyzed and unhydrolyzed crude protein (0.05 mg/mL) of functional beverage formulated from mature seeds of *Phaseolus vulgaris* L.

Crude protein from functional beverage formulated from *Phaseolus vulgaris* L. and its hydrolysates	% Cholesterol micellar inhibition* (Mean)	SD
Functional Beverage (unhydrolyzed)	14.89[e]	0.76
Peptic Digest (1 hour)	24.31[bc]	1.00
1-hour combined enzymes digest	21.25[cd]	2.28
6-hour combined enzymes digest	27.38[b]	1.65
12-hour combined enzymes digest	19.71[d]	0.76
24-hour combined enzymes digest	22.12[cd]	1.00
Cholestyramine (positive control)	60.45[a]	1.00

*All data are the means of three measurements. Tukey's HSD test with one-way ANOVA at a 95% confidence interval reveals significant differences between letter superscripts of means at $p<0.05$; SD: Standard Deviation

in cooked cowpea beans (39.8%), indicating that cooking has enhanced the release of peptides that can suppress cholesterol solubility.

During digestion, dietary cholesterol interacts with bile acids to create micelles. This interaction enhances the solubility and absorption of cholesterol in the intestines. Consequently, formation of micelle can be suppressed to reduce cholesterol (Chen *et al.* 2021). In bovine milk, β-lactoglobulin tryptic hydrolysates (Ile-Ile-Ala-Glu-Lys) were observed to suppress the incorporation of cholesterol into the micelles. This regulates the absorption of cholesterol in the jejunum (Nagaoka *et al.* 2001). Cholesterol not incorporated into the micelles forms aggregates and is unable to be absorbed. As a result, it travels into the colon to be expelled in the feces as organic matter (Zhang *et al.* 2012). This may be because of the composition and amino acid sequence in peptides. For instance, amino acids with hydrophobic properties bind competitively with bile acid and can modify the structure of cholesterol, therefore disrupting cholesterol micelle formation and limiting the absorption of exogenous cholesterol (Chen *et al.* 2021).

Notably, the 6-hour combined enzyme demonstrated the highest % cholesterol micellar inhibition compared to the unhydrolyzed beverage and single enzyme peptic digest. This could be related to the combination of enzymes used in digestion which demonstrated synergistic effect enhancing the generation of bioactive peptides (Akbarian *et al.* 2022). Protein digests bioactivities could also be affected by the proteases used, enzyme/substrate ratio, processing conditions, sequences of peptides, and amino acids generated during hydrolysis due to specificity of enzymes (Mangussad *et al.* 2021; Nasri 2017).

The protein hydrolysates produced through enzymatic digestion compete with cholesterol in forming cholesterol micellar mixture and interacting with bile salts. Polar peptides may interact with micellar hydrophilic bile salts and consequently reduce solubility of micellar cholesterol, whereas nonpolar peptides bind with cholesterol, impeding cholesterol solubilization into the micelles (Jiang *et al.* 2020). The disrupted formation of micellar cholesterol solution leads to cholesterol aggregation which could be eliminated from gastrointestinal tract via feces (Upadhyay *et al.* 2021).

CONCLUSION

Phaseolus vulgaris L. could be exploited for the development of functional beverages. The protein hydrolysates from the formulated functional beverage of common bean seeds can potentially prevent hypertension and hypercholesterolemia. Understanding the properties of proteins derived from common beans is essential for incorporating the proteins as ingredients in the processing of highly nutritious beverages or bean-based food products. Furthermore, in vivo studies are needed to explore more detail mechanism of *Phaseolus vulgaris* L.

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DECLARATION OF CONFLICT OF INTERESTS

The authors declared that they have no conflict of interest.

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Research Article

Effectiveness, Consumer's Perception, and Behavior Towards Healthier Choice Logo on Indonesian Instant Noodles in Jakarta

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ABSTRACT

This study was conducted to identify the availability of instant noodle products with and without the Healthier Choice (HC) logo that reflects Indonesian instant noodles nutrition labelling uptake by the food industry as well as to investigate the logo's effectiveness in influencing customers to choose healthier noodle products and consumers' perception and behavior toward the HC logo. Quantitative research consists of a market survey on HC logo usage in 120 instant noodle variants with different brands and an online consumer survey with a voluntary sampling technique involving 458 consumers aged 18–68 years residing in Jakarta. Nutritional information data (total fat, saturated fat, sodium and sugar) in two groups of products (HC and Without HC (WHC) groups) was analyzed using t-test, while the consumer survey data was analyzed using t-test and ANOVA. The confidence level used in statistical analysis was 95%. Of only eleven products that featured the HC logo on the packaging, one did not fulfill the criteria. The t-test showed no significant differences between products "without HC logo" and "with HC logo" groups in all nutrition content except for sodium. The HC logo showed significant difference in effectiveness for different gender and age groups, it was better for women than man, and older (43−58 and 59−68) more effective than younger (16−26 and 27−42). The consumer perception and behavior was more affected the age group 43−58 and 59−68 than 16−26 and 27−42, the level of education group in elementary, junior, and senior high school also post graduate group more influenced than pre-university and bachelor, consumption pattern moderate and frequent better than seldom consumption. Further, the consumer behavior also more influenced all income level group except >IDR20 million group.

INTRODUCTION

is to reduce premature deaths caused by Non-Communicable Diseases (NCDs). In Indonesia, NCDs such as heart diseases, cancer, diabetes, and chronic respiratory diseases account for 73% of all deaths (WHO 2022). The World Health Organization (WHO) has published a global action plan to address the NCD issue. The global action plan includes regulation of Front-of-Pack (FoP) nutrition labeling to decrease population consumption of total fat, saturated fat, sodium, and sugar (Vargas-Meza *et al.* 2019), help consumers decisions towards healthier food choices, and encourage the industry to reformulate products towards healthier options (Kanter *et al.* 2018). FoP nutrition labeling has been implemented worldwide through government policies, such as The Choices Program logo or the Healthy Choices logo, which was first endorsed by European governments (Kanter *et al.* 2018).

The Indonesian FDA issued nutrition labeling regulations in Indonesia to address the NCD issue, but consumers are having difficulties interpreting the information about nutritional value on Back-of-Package (BoP). In 2021, The Indonesian FDA introduced the Healthier Choice scheme as a FoP voluntary label starting with instant noodles and ready-to-drink products in 2019 (FDA 2021a). Instant noodles are Indonesia's most consumed food product (14,260 billion servings per year) in 2022 (WINA 2022). Instant noodle production uses raw materials and frying technology, which can increase the nutrition content of total fat, saturated fat, and sodium (May & Nesaretnam 2014). Those nutrients potentially increase NCDs risk; according to Syauqy *et al.* (2022), instant foods increase hyperglycemia in middle-aged (45–59 years) in Indonesia. Regulation is needed to push consumers to choose healthier instant noodles (Istiqomah *et al.* 2021) to reduce the NCDs in the Indonesian population.

Effectiveness is the degree to which something is successful in producing a success result. The effectiveness of the HC logo can be seen from the consumer's knowledge and awareness regarding the availability of the HC logo in noodle product also the confidence level towards Indonesian FDA (Vargas-Meza *et al.* 2019; Izzati *et al.* 2022). Perception is the ability to see, hear, or become aware of something through the senses, whereas behavior is the way in which one acts or conducts oneself, especially toward others. In this research, perception towards HC logo observed from the easiness of the logo to be understandable and shown in noodle product packaging, the impact of the HC logo in choosing the healthier noodle product, and the credibility of the HC logo (Fatimah *et al.* 2019; Fialon *et al.* 2022; Izzati *et al.* 2022). Consumer behavior toward HC logo define from the transformation of the consumption pattern that cause by the HC logo, the influence of the non-communicable diseases, effect of price and taste of the noodle product, and the impact of HC logo towards purchase decision (Méjean *et al.* 2013; Maemunah 2020; Izzati *et al.* 2022).

After four years of implementation, there is a need to examine the update on the HC logo uptake in instant noodle products, compare the nutritional values (total fat, saturated fat, sodium, and sugar) among the group of products with the HC logo (HC) and without the HC logo (WHC), as well as to evaluate the effectiveness, consumer perception, and behavior towards the HC logo on instant noodles. This research is needed for the government and policymakers to evaluate the effectiveness of the HC logo and the influence of the HC logo on consumer perception and behavior, which can improve their eating habits. The effectiveness of the HC logo in affecting consumer perception and behavior is essential to ensure that the objective of regulation about the HC logo is well achieved.

METHODS

Design, location, and time

The study was conducted in Jakarta, Indonesia, for five months (January to May 2023). The research consisted of: 1). A market survey to identify the availability of instant noodle products with and without the HC logo, and; 2). A consumer survey to determine the effectiveness of the HC logo in influencing customers to choose healthier instant noodles, as well as to understand the consumer perception and behavior toward the HC logo. The study protocol was approved by the Research Ethical Commission of IPB University for using respondents as the subject in document 900/IT3.KEPMSM-IPB/ SK/2023.

Sampling

The market survey used instant noodles available in the Jakarta retail market and in online stores as the sample of this research. The inclusion criteria were instant noodles produced locally, had Indonesian FDA approval, made with wheat flour, and provided nutritional information. The market survey obtained 19 brands (A to Z) with 120 products reflecting specific brands' flavors .

The eligibility criteria for the consumer survey were defined, including being aged 15−68 years, residing in Jakarta, and be an instant noodle's consumer. If the respondent did not meet one of the criteria, they would be excluded from the research. The respondents were chosen using a voluntary quota sampling technique. Taking into account of Jakarta's population size aged 15−68 years old, and employing the confidence level of 95% and a sampling error of 5%, the minimum sample size necessary for the study was calculated to be 400.

Data collection

Market survey. This stage involved collecting the instant noodles samples and categorizing them into two groups based on their eligibility to bear the HC logo: without the HC logo (WHC) and with the HC logo (HC). Serving size and nutritional information, including total fat, saturated fat, sodium, and total sugar were based on the information displayed on the package and further calculated per 100 g. The nutrient contents were compared between HC and WHC to determine the differences in those groups based on the information displayed on the package.

Consumer study. This research used an online survey platform, Google Forms, and the participants were recruited via the researcher's network (WhatsApp). There were three sections in the questionnaire identifying the effectiveness, consumer perception and consumer behavior toward the HC logo. The respondent would answer the question in Likert Scale. All respondents had read the consent form and agreed to participate before starting the survey.

Data analysis

Data analysis was performed using Statistical Package for Social Sciences (SPSS) version 25 (SPSS Inc, USA). Pearson product-moment and Cronbach's alpha were used to determine the validity and reliability of the questionnaire. The questionnaire was based on an ordinal 5-point Likert scale that needed to be converted to an interval scale using the Z-table normal distribution method (Santoso 2015). An independent sample T-Test was used to determine the differences in four nutrients between WHC and HC groups and to analyze the differences in effectiveness, consumer perception, and behavior in gender groups. Analysis of Variance (ANOVA) and Tukey post hoc test were used to see the age, level of education, income, and instant noodle consumption group variance in the effectiveness, consumer perception, and behavior. Pearson's Chi-Square Test was used to determine the relationship between noodle consumption and income level. Data analysis was assessed with a 95% confidence interval and a 5% significance level, and statistical significance was accepted as $p<0.05$

RESULTS AND DISCUSSION

Market survey

Based on Annex IV of Indonesian FDA Regulation No. 26 of 2021 on Nutrition Labeling on Processed Foods (FDA 2021b), the criteria for healthier "instant noodles" were containing total fat ≤20 g/100 g and sodium ≤900 mg/ 100 g. Even though sugar and saturated fat were not the criteria for the HC logo uptake, these need to be analyzed because those nutrients might related to the NCD in Indonesia. Another fact was that the HC logo in Singapore uses saturated fat as the criteria for the HC logo application.

Table 1 showed a total of 120 samples of instant noodle products. Of 109 products (91%) categorized as WHC, 103 products were not eligible and 6 products were eligible to use the logo. From 11 products (9%) that used the HC logo, 10 products were qualified to feature it (all products from brands E, G, I, M, S, and 2 products from brand H), while 1 product was ineligible to use the HC logo (1 product from brand H due to sodium exceeding 900 mg/100 g). Despite being introduced four years ago, only 9% of Indonesian instant noodle products used the HC logo. The low uptake was suspected to be due to the voluntary nature of the HC logo; similar results were shown in a study of the voluntary HSR (Healthy Star Rating) uptake in New Zealand, where only 5.3% of products displayed the HSR logo two years after it was introduced (Ni Mhurchu *et al.* 2017).

Nutritional values (total fat, saturated fat, sodium, and sugar in 100 g portion size) of instant noodles products were grouped into 2: "with the HC logo" (HC) and "without the HC logo" (WHC). HC group included products that featured the HC logo on their FoP nutrition labeling. On the other hand, the WHC group consisted of products that did not use the logo, even if the product met the requirement. The result showed no significant differences between the two groups in all elements except sodium ($p<0.05$) (Table 2).

Table 1. Indonesian instant noodle products without and with thehealthier choice logo and its eligibility

Product category	Product amount		
	Eligible	Not eligible	Total product
HC	10	1	11
WHC	6	103	109

HC: Healthier Choice; WHC: Without Healthier Choice

Table 2. Range and average of nutritional composition for each brand of Indonesian instant noodles

Brand code	n	Portion size (/100 g)			
		Total fat (g)	Saturated fat (g)	Sodium (mg)	Sugar (g)
Without the HC logo (WHC)					
A	8	15.29–26.25	5.88–13.75	752.94–2042.86	0.00–5.88
B	3	23.53-25.00	7.50–11.25	1,200.00–1270.59	6.25–9.41
C	1	7.69	2.00	1,338.46	4.62
D	13	16.00–20.00	6.67–9.09	320.00–2,740.00	1.33–8.24
F	33	14.29–25.88	7.14–12.00	877.78–1,993.33	0.00–10.67
G	2	3.00–6.00	0.00–2.00	1,630.77–1,753.85	5.00–6.00
I	3	5.00-11.00	3.00–6.00	1,328.77–1,900.00	5.00–6.00
J	3	10.00–14.00	4.00–6.00	830.99–2,000.00	2.00–9.00
K	4	18.00–20.00	7.00–10.00	2,433.33–3,035.71	0.00–3.57
L	4	15.00–18.00	7.00–9.00	1,039.47–1,444.44	4.00–7.00
M	2	9.00–13.00	4.00–6.00	1,213.00–1,363.00	3.00–4.00
N	3	18.00–21.00	10.00	1,242.86–2,066.67	3.00–7.00
O	4	17.00–22.00	8.00–11.00	1,030.77–1,283.33	2.00–8.00
P	5	16.00–20.00	7.00–10.00	1,175.00–2,015.87	4.00–7.50
Q	17	14.44–21.05	5.75–9.33	627.91–1,800.00	1.61–7.78
R	4	17.00–31.00	9.00–14.00	1,157.14–1,373.33	3.08–6.00
Range		3.00–31.00	0.00–14.00	320.00–3,035.71	0.00–11.00
With the HC Logo (HC)					
E	1	5.00	4.38	500.00	6.25
G	1	7.50	3.13	737.50	8.75
H	3	1.00–4.00	0.00–2.00	539.47–925.53	1.33–4.00
I	3	5.00–9.00	2.00–4.00	787.50–847.06	4.00
M	1	13.33	5.56	633.33	8.89
S	2	10.00–19.00	3.00–9.00	737.50–862.50	5.00–8.00
Range		1.00–19.00	0.00–9.00	500.00–925.53	1.33–8.89
WHC Mean±SD		17.66±4.28[a]	8.39±2.35[a]	1,406.77±464.94[a]	4.95±2.33[a]
HC Mean±SD		7.54±5.11[a]	3.36±2.46[a]	740.75±134.63[b]	5.00±2.79[a]
p		0.376[a]	0.986[a]	0.021[b]	0.469[a]

a, b: The number in the mean and p-value column with the same letter indicated no significant differences in the significance level of 5%; n: the amount of variants in each brand; HC: Healthier Choice; WHC: Without Healthier Choice; SD: Standard Deviation

According to WHO recommendations, the maximum daily fat intake should not exceed 30% of the Total Energy intake (TE). If the energy requirement was 2,000 kcal/day, the maximum total fat intake should be less than 67 g/day (30% TE) (WHO 2020); in line with this, the Indonesian Ministry of Health suggested that total fat consumption should be less than 67 g/day (MoH RI 2019). The mean total fat content in the WHC group was 17.66±4.28 g/100 g, which was higher than the HC group (7.54±5.11 g/100 g). However, the total fat in both groups was lower than the WHO or Ministry of Health recommendation, and the total fat content between the two groups was not significant (p>0.05).

WHO recommends consuming less than 10 g/100 g of saturated fat daily, while the Indonesian Ministry of Health did not set a daily recommendation. Overall, this study showed that the saturated fat content of instant noodles in both HC (3.36±2.46 g/100 g) and WHC groups (8.39±2.35 g/100 g) was less than the WHO recommendation for daily consumption despite those nutrients not being the requirement in the HC logo criteria.

The Indonesian Ministry of Health stated that adolescents should limit sodium intake to up to 2000 mg/day (MoH RI 2019), the same as the WHO recommendation (WHO 2020). This study showed that soup-based noodles had higher sodium than fried noodles; since their seasoning must be diluted in water, adding salt will make it tastier. This result was in line with the study of Istiqomah *et al.* (2021) that Indonesian soup-based instant noodles tended to have higher sodium content than fried-based instant noodles, with the mean of both local instant noodle-based products being 1,258.22 mg/100 g from 305 products which was analyzed in 2019 to 2020 (Istiqomah *et al.* 2021). In this study, only eleven products had a sodium content of more than 2,000 mg/100 g, but the mean of WHC group (1,406.77±464.94 mg/100 g) and HC group (740.75±134.63 mg/100 g). Afterward, the mean sodium content in the HC group was significantly different (p<0.05) than in the WHC group, the significant difference is due to lower sodium content is mandatory requirement to obtain the HC logo criteria. Regarding nutritional composition per serving, it was generally observed that the sodium content was within the recommendations set by the Indonesian Ministry of Health. However, the limit was considered higher than other healthier choice criteria in the Southeast Asian countries, except Thailand which had a sodium limit of ≤2,000 mg/100 g.

WHO and the Indonesian Ministry of Health recommend that sugar intake per day should be less than 50 g/day; however, consuming less than 25 g of sugar/per day was preferable to increase public health (WHO 2020). This study showed that the mean sugar content in the two groups, WHC (4.95±2.33 g/100 g) and HC group (5.00±2.79 g/100 g), were not significantly different (p>0.05). The HC group had slightly higher sugar content than the WHC group. Adding sugar might make the noodle HC group tastier to compensate for lower sodium content (Istiqomah *et al.* 2021). However, in this study, the sugar content of both groups of instant noodles was within the limit set by the Indonesian Ministry of Health and was not included in the HC logo criteria for instant noodles product. Beverage and sweet snack products were identified as the primary sources of sugar intake for school-age children, adolescents, and adults (Andarwulan *et al.* 2021). Beverage products required 6 g/100 mL to utilized Healthier Choice logo, while the sweet snack products (biscuit, cookies, wafer) required sugar 20 g/100 g (FDA 2021b). Instant noodles were not considered the major contributors to the sugar intake (Andarwulan *et al.* 2021).

Consumer survey

The respondent profiles from the consumer survey are presented in Table 3. The HC logo was more effective in women than men (gender group). According to Lassen *et al.* (2016), adult Danish customers showed that women pay more attention to nutrition while men choose food based on price. The HC logo was also more effective among respondents aged 43−58 and 59−68 age than aged 18−26 and 27−42 (p-value<0.05). In a previous study, consumers aged 50−65 showed concern and higher effectiveness in FoP nutrition labeling than those aged 20−34 in the United States (Roark *et al.* 2022). The limitation of the current work should be acknowledged: one of the evaluations was the age group, which has a wide range. The HC logo was considered the most straightforward logo to understand, however it did not give thorough information about the products. Therefore, logo affected the lower income more, as demonstrated in a similar study conducted in Mexico (Vargas-Meza *et al.* 2019). In the current study, the HC logo effectiveness

Table 3. Effectiveness, consumer perception, and behavior toward the healthier choice logo

Respondent criteria	n (n=458)	Mean±SD		
		Effectiveness	Perception	Behavior
Gender				
Man	195	2.17±0.62[a]	2.11±0.59[a]	1.56±0.67[a]
Women	263	2.32±0.53[b]	2.20±0.57[a]	1.58±0.63[a]
Age				
18–26 years old	50	2.22±0.59[a]	2.00±0.50[a]	1.27±0.56[a]
27–42 years old	232	2.15±0.60[a]	2.03±0.64[a]	1.30±0.58[a]
43–58 years old	132	2.35±0.55[b]	2.32±0.48[b]	1.98±0.50[b]
59–68 years old	44	2.55±0.32[b]	2.50±0.24[b]	2.13±0.38[b]
Level of education				
Elementary, Junior & Senior high School	54	2.37±0.72[a]	2.34±0.64[a]	1.78±0.67[a]
Pre-University & Bachelor	283	2.22±0.56[a]	2.11±0.58[b]	1.53±0.65[b]
Post graduate	121	2.36±0.51[a]	2.24±0.45[a]	1.60±0.59[a]
Level of income				
≤IDR5 million	70	2.31±0.69[a]	2.29±0.63[a]	1.72±0.65[a]
IDR5–10 million	190	2.25±0.55[a]	2.12±0.57[a]	1.53±0.63[a]
IDR10–15 million	98	2.22±0.50[a]	2.13±0.54[a]	1.62±0.66[a]
IDR15–20 million	46	2.24±0.66[a]	2.22±0.59[a]	1.66±0.64[a]
IDR20 million	54	2.29±0.60[a]	2.11±0.58[a]	1.36±0.60[b]
Consumption Pattern				
Seldom (1–2x/week)	293	2.20±0.59[a]	2.09±0.59[a]	1.40±0.61[a]
Moderate (3–5x/week)	118	2.34±0.54[a]	2.27±0.53[b]	1.85±0.58[b]
Frequent (6–7x/week)	47	2.40±0.58[a]	2.32±0.57[b]	1.97±0.59[b]

[a, b]: Same letter in the same column indicated no significant differences in the confidence level of 95% ($p>0.05$)
IDR: Indonesian Rupiah
SD: Standard Deviation

was not influenced by the level of education, income, or consumption pattern (p>0.05). But, higher effectiveness of the HC logo was shown in women and respondent aged 43−68, they had better knowledge and awareness about HC logo, higher trust to the Indonesian FDA and aware of the purpose of the HC logo implementation.

In contrast to effectiveness, gender groups did not affect the consumer perception toward the HC logo on instant noodles (p>0.05). Similar to effectiveness, age group affected the consumer perception toward instant noodles more in aged 43−58 and 59−68 group compared to 18−26 and 27−42 group (p<0.05). In line with previous research, older Brazilian respondents believed that consuming healthier food could reduce the risk of developing NCDs in older age (Marsola *et al.* 2020). Respondents with pre-university and bachelor degree had the lowest score on perceptions towards the HC logo and were significantly different from all education groups (p<0.05). Previous study showed that the most uncomplicated logo, such as the green tick logo, affects the lower education group (Guthrie *et al.* 2015); interestingly, in our study, elementary, junior, and senior high school groups had higher scores among other education groups. Meanwhile, this research showed that income level did not influence consumer perceptions of the HC logo, even though another study stated that implementing the HC logo increased Malaysian consumers' willingness to buy; despite its higher price, they would choose products with the HC logo (Fatimah *et al.* 2019). Moderate and frequent consumption patterns of instant noodles affected consumer perceptions towards the HC logo compared to "seldom" consumption of instant noodles (p<0.05). Consistent with this, consumers who consume instant noodles more frequently were more concerned about choosing healthy instant noodles, and some people reduced their instant noodle consumption. In this research, consumer better perception towards HC logo was shown in respondent aged 43−68, with lower and very high education group (elementary, junior, and senior high school also post graduate), and among those who had moderate and frequent consumption pattern. They believe that HC logo was trusted, credible and had positive added value in noodle product packaging. They also noticed the HC logo message, realized the HC logo was associate with the nutritious and healthier product, and the HC logo could encourage consumer to choosing the noodle healthier product.

Similar to perception, gender did not affect consumer behavior in choosing instant noodles with the HC logo (p>0.05). Meanwhile, aged 43−58 and 59−68 group were better than 18-16 and 27−42 group at utilizing the HC logo to influence their choice (p<0.05). As shown in previous studies, the FoP nutrition schemes influenced behavior and promoted healthier diet patterns among consumers over 40 years in Portugal (Silva *et al.* 2022). Pre-university and bachelor groups were the group that significantly influenced by the HC among all education groups (p<0.05). One theory might explain why the elementary, junior, and high school education group had a higher score in consumer behavior towards the HC logo because the design was too simple and cannot provide a detailed product such as nutritional information; meanwhile, higher education wants more information when the lower education preferred the most uncomplicated logo like HC logo (Méjean *et al.* 2013). The group with the level of income >IDR20 million significantly different in consumer behavior compared to other income groups (p<0.05) and their consumption score was the lowest among the other income groups. A simple reason might be that this group had the most significant food options; thus, instant noodles were not a common choice. A previous study in Brazil showed that high-income consumers did not mind food prices, rarely buy fast food products (Marsola *et al.* 2020), and prefer healthy products (Vos *et al.* 2022). This study showed that the behavior of consumers with "moderate" to "frequent" consumption patterns was more influenced by the HC logo (p<0.05) than those who rarely consume instant noodles; this could be explained by previous research that there was an increase in consumer intention to purchase healthier products after the COVID-19 pandemic in Saudi Arabia (Hesham *et al.* 2021). To recap, the HC logo statistically affects the behavior of consumers in all groups except gender. In this research, consumer behavior associated with HC logo was shown in respondent aged 43−68, and among respondents with lower education group and very high education (lelementary, junior, and senior high school and post graduate), all level of income except those earned more than 20 million rupiah, and among consumers with moderate and frequent consumption pattern. They bought and consumed the instant noodle with the HC logo, changed their mind because the HC logo and still bought the product even though the price was higher, but not all affected respondent want to sacrifice the taste of the instant noodle even when there was HC logo in the packaging.

Some limitations should be considered regarding using these data to monitor the implementation of the HC logo system, especially on instant noodles in Indonesia. First, product collection occurred in two supermarket stores in Jakarta, and the marketplace might not capture products that are maybe available in other stores. Second, despite listing saturated fat content on the Nutrition Facts Regulation was mandatory, the data available were only for 111 from 120 products. Third, the initial analysis was conducted utilizing data per 100 g. However, it should be noted that the actual serving sizes of these products fall below the 100 g threshold, and each product had a different serving size. Finally, because the number of products displaying the HC logo in the database was small (11 products out of 120 local products), caution should be exercised in interpreting data and drawing conclusions pending wider uptake.

CONCLUSION

One hundred-twenty Indonesian instant noodle products consisted of 91% (109 products) of the WHC group and 9% (11 products) of the HC group. There was one product displaying the logo was found to be not eligible to use the logo which shows lack of supervision after the logo was authorized for the product.

The HC logo was more effective for women aged 43−58, and 59−68 than men and younger (18−42). Meanwhile, consumer perception toward instant noodles and consumer behavior in age group 43−58 and 59−68 was more affected than aged 18−26 and 27−42. Elementary, junior, senior high school and post graduate group was more influenced than pre-university and bachelor. Moderate and frequent consumption was more affected than seldom consumption. Further, all group in income level was more influenced in behavior toward instant noodles except in income >IDR20 million) both the market survey and consumer survey in this study, further research on HC logo effectiveness could use larger samples of products and broader respondents to better represent the Indonesian population.

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DECLARATION OF CONFLICT OF INTERESTS

The authors have no conflict of interest.

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