



## Effectiveness of Calcium-Fortified Thai Traditional Cracker Using Cricket Protein on Biochemical Marker of Bone Health and Bone Mineral Density

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### ABSTRACT

**Background:** Thailand is one of the countries facing a high prevalence of low bone mineral density (BMD) due to inadequate calcium (Ca) intake. Using crickets as alternative protein sources is fascinating for nutritionists because of their high biological value and cost-efficient production. This study aims to investigate the effectiveness of Ca-fortified Thai traditional crackers using cricket protein. **Methods:** Four formulas of Ca-fortified crackers were prepared with different amounts of Ca based on the percentage of recommended Ca in adults for one portion (15%, 25%, and 50% per 250 mg of cracker dough) before conducting a sensory evaluation with 30 participants. The selected acceptable Ca-fortified cracker formula (50% of Ca by RDA per portion) was further used in an intervention study with another group of 40 participants (intervention group=20 and control group=20). The participants in the intervention group were asked to consume 250 g of crackers/day of 50% of RDA per portion formula for 12 weeks to compare bone health parameters with the control group. **Results:** Results revealed that BMD values of wrist and ankle bones in the intervention group were significantly higher than those in the control group ( $P=0.004$  and  $P<0.001$ , respectively), and alkaline phosphatase (ALP) levels in the intervention group were significantly lower than those in the control group ( $P=0.04$ ) at endpoint. The results also found a positive correlation between Ca intake and BMD parameters among all participants ( $P<0.001$ ). **Conclusion:** The developed Ca-fortified Thai traditional crackers using cricket protein is preliminarily effective in improving bone health conditions among the participants.

### Introduction

Low bone mineral density (BMD) is one of the major public health problems globally, caused by bone resorption and inadequate intake of calcium (Ca) in the long term (Akkawi and Zmerly, 2018, Pouresmaeli *et al.*, 2018). Declining BMD leads to bone complications, particularly osteopenia and osteoporosis, which

impact patients' quality of life by making them suffer and struggle with daily activities (Gold *et al.*, 2019). There are reports on the prevalence of people with low BMD. For example, 10 million US adults are diagnosed with osteoporosis, and an estimated 15 million adults have osteoporosis in Japan (Clynes *et al.*, 2020, Iki, 2012). In Thailand,

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the prevalence of low BMD is increasing due to population aging and inadequate Ca intake among younger individuals. A previous study indicated that 89% of older Thais living in healthcare residences were found to have osteopenia (Assantachai *et al.*, 2006), and there is an increased risk of low BMD development among menopausal women (Amphansap *et al.*, 2021). It is well known that Ca is essential for human bone formation. A study reported that inadequate Ca intake leads to low serum Ca levels (Kim *et al.*, 2018), which are positively related to low BMD values (Sun *et al.*, 2021). Previous research revealed that the average amount of Ca intake among Thai adults is 300 mg/day, which is far from the recommended daily amount of 900 mg/day (Pongchaiyakul *et al.*, 2008). The potential factors contributing to this nutritional problem are food culture with low milk consumption, and milk allergies and lactose intolerance that cause people with these conditions to avoid milk and milk products, which are abundant sources of Ca (Hodges *et al.*, 2019). Hence, promoting milk consumption and developing alternative food products that are good sources of Ca are important to encourage adequate Ca intake among people and lower the risk of BMD problems in the long term (Tai *et al.*, 2015).

Protein is well known as an essential nutrient for maintaining optimal human physiological functions such as growth, hormone production, and muscle building. Therefore, adequate protein intake is important for all people. Nowadays, insect protein is an alternative protein source that fascinates professionals in nutrition due to its high biological value (Hermans *et al.*, 2021). Additionally, using insect protein supports food security by replacing high-cost protein production such as pork, beef, and chicken, which require high demand for public utilities and contribute to greenhouse gas emissions (Skrivervik, 2020). Hence, the use of insect protein is beneficial for the bioeconomy, especially crickets, which are popular as a protein source due to their ease of rearing, low space requirements, low utility and feed costs, and high-quality protein yield (Murugu *et al.*, 2021). Previous research has found benefits of cricket

protein on gut microbiota, including reducing TNF-alpha, which may help reduce inflammation in the body (Stull *et al.*, 2018). Although clinical research related to insect protein is still limited as it is considered novel, foods with insect protein sources, especially crickets, are considered a future food trend (Kim *et al.*, 2019). Thai traditional rice crackers are a type of Thai food product that has been around for many eras.; they are popular, easy to eat, and have a long shelf life compared to general dairy snack products (Maisont *et al.*, 2021). Given the data on insufficient calcium intake among the Thai population leading to the risk of osteoporosis in old age, as well as the benefits of cricket protein mentioned, this research aims to develop calcium-fortified Thai traditional cricket protein crackers as a nutritional innovation to serve as an alternative food for those with insufficient calcium intake and investigate its effectiveness on participants' BMD values and biochemical marker of bone health.

## Materials and Methods

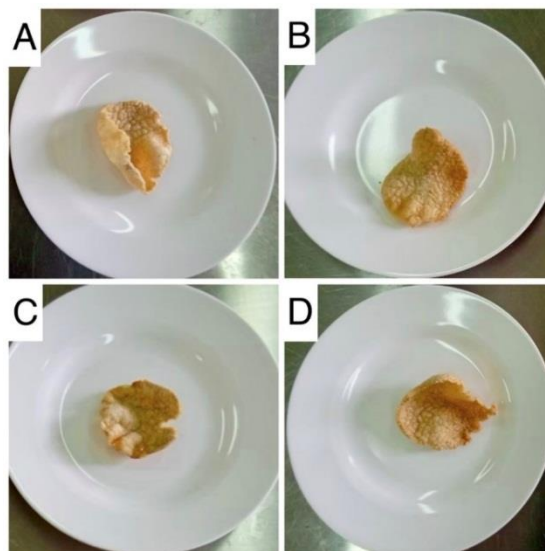
This randomized-controlled trial study was conducted from June 1, 2023-30 May 2024, involving general people to live in Chonburi province, Thailand.

### *Calcium-fortified cricket protein cracker product*

In developing the calcium-fortified cricket protein cracker dough, the recipe and preparation method was adapted from the information published on the Internet, as it can be easily applied in households. The amounts of various ingredients (such as tapioca flour, salt, pepper, etc.) was adjusted as appropriate during preparation. The meat ingredient was replaced with a commercial cricket protein powder product made from the *A. domesticus* strain, as it provides high protein and low-fat content. Additionally, calcium citrate powder was added to the crackers, as it is a form of calcium that dissolves well in water, making it suitable for mixing into the cracker dough. It is also one of the forms of calcium with high bioavailability in human body (Hanzlik *et al.*, 2005). The total amount of calcium added

(including the calcium from the cricket protein powder) per 250 grams of raw cracker dough (before frying) were calculated to provide various percentages of the daily requirement for adults:

15% (150 mg), 25% (250 mg), and 50% (500 mg), along with a control formula without calcium fortification. This resulted in a total of 4 formulas (Figure 1).



**Figure 1.** Four formulas of the developed crackers with cricket protein samples (A; control, B; 15% of Ca by RDA per serving, C; 25% of Ca by RDA per serving, and D; 50% of Ca by RDA per serving).

The crackers from each formula were then fried in palm oil and subjected to sensory evaluation by 30 participants using 9 level-hedonic scale to select the formula with the highest acceptance and evaluation scores for further research (Figure 2). The participants were recruited following inclusion criteria-healthy Thai individuals between the ages of 18 and 45 who can read and write Thai-were chosen for the study in order to do the sensory evaluation on the four cracker recipes that had been prepared. Individuals with a medical history of food allergies, mental disease, color blindness, or any other ailment that impairs their sensory

perception were excluded from consideration. Terrible=1 point, very bad=2 points, bad=3 points, just a little bad=4 points, maybe good or maybe bad=5 points, just a little good=6 points, good=7 points, very good=8 points, and great=9 points was the scoring method used for the sensory evaluation questionnaire. The cracker formula that received an average overall satisfaction score greater than 7.00 points was considered to have passed the satisfaction level by participants (Wichchukit and O'Mahony, 2015) and was considered for use in the next phase of research.



**Figure 2.** The nine level-hedonic scale.

### ***Physico-chemical and Ca content analysis***

Based on sensory evaluation results, the selected two cracker formulas were further performed by comprehensive analysis: the control formula and one Ca-fortified formula that received satisfactory and acceptable ratings from participants. These samples underwent extensive testing at the Thailand National Food Institute under Thailand's Ministry of Industry. The analysis included ash content: measured using an in-house method T924 adapted from Association of Official Agricultural Chemists (AOAC, 2019) 923.03, Ca levels: determined by in-house method T9152 based on AOAC (2019) 984.27, color parameters: assessed using a Hunter Lab Color Quest XE device to measure L\*, a\*, and b\* values, moisture content: evaluated using in-house method T923, derived from AOAC (2019) 925.10, and protein content: quantified through in-house method T927, following AOAC (2019) 991.20. This thorough analysis provided a comprehensive profile of the crackers' composition and nutritional value.

### ***Effectiveness of the selected Ca-fortified cracker formula on bone health parameters***

After enrollment, another group of 40 participants was determined by G\* Power sample size calculation and guideline by previous study (El-Hagrassy *et al.*, 2018), who were then recruited and randomly divided into a control group of 20 and an intervention group of 20. These participants were recruited to test the effects of consuming the Ca-fortified cricket protein crackers that had passed the sensory evaluation test using the following inclusion criteria: being between 20-45 and having Thai ethnicity and nationality, and exclusion criteria: having chronic diseases, gastrointestinal disorders, currently taking herbal products or dietary supplements, history of cow's milk allergy, being pregnant or breastfeeding babies, those with bone fractures, acute or chronic kidney failure, endocrine system diseases, currently taking any dietary supplementations, and taking medications that affect bone density (such as bisphosphonates, vitamin D). After all the

participants signed the consent form in the provided room at Faculty of Allied Health Sciences, Burapha University, Chonburi, Thailand, they were then interviewed about their eating habits using a 24-hour recall questionnaire to calculate nutrient intake, calcium intake, and daily exercise information. Blood samples were collected to measure serum alkaline phosphatase (ALP) levels which is considered one of the biochemical markers of bone resorption (Cheng and Zhao, 2023). Body composition and resting energy expenditure were measured using an InBody 270 body composition analyzer (InBody Inc., Seoul, South Korea). Bone density (wrist and ankle) was measured using a dual-energy X-ray absorptiometry (DEXA) device (Osteo checker, Ampall Co Ltd., Seoul, South Korea).

After completing these steps, the control group participants were scheduled for another appointment 12 weeks later to measure their body composition and bone mass again at the endpoint. For the intervention group, participants were asked to continuously consume the selected calcium-fortified cricket protein crackers every weekday at lunchtime for 12 weeks. The amount of Ca obtained in consumed crackers was calculated to provide about 50% of the daily calcium requirement for adults, as this is the recommended amount of calcium per meal for optimal absorption (Garg and Mahalle, 2019). Adherence to the dietary intervention was monitored and evaluated through the LINE messaging application, where participants were required to submit photographs documenting each instance of calcium-fortified cracker consumption. At the end of the 12<sup>th</sup> week (endpoint), participants were invited to the same provided room for a dietary history interview, blood sample collection to measure ALP levels, body composition measurement, and bone density measurement. In addition, all participants were interviewed to collect their dietary habits and analyze their nutrient intake using a nutritional software package, INMUCAL-Nutrients version 4.0, developed by the Institute of Nutrition, Mahidol University, Thailand. The Institutional



Review Board for Research Ethics at Burapha University reviewed and granted approval for this study's methodology (approval number IRB1-091/2566).

### **Ethical consideration**

This study obtained the ethical approval by the Institutional Review Board for Research Ethics at Burapha University (approval no. IRB1-091/2566)

### **Data analysis**

Sensory evaluation scores and parameters of bone health, including ALP level, t-score, and BMD value were presented as mean  $\pm$  SD. Shapiro-Wilk test was used to determine normality of the data distribution. Tukey's Honestly Significant Difference test was used for multiple pairwise comparisons of satisfaction scores among different formulas in each aspect. An independent t-test was used to determine the difference between bone health parameters between groups, and the Pearson correlation coefficient was used to determine the amount of Ca intake and BMD values using Statistical Package for the Social Sciences (SPSS Inc., Chicago, IL), version 21.0. A statistically significant difference was deemed at  $p < 0.05$ .

## **Results**

### **Sensory evaluation of the developed Ca-fortified cracker with cricket protein**

The sensory evaluation questionnaire was used to determine the participants' acceptability and overall satisfaction with the four cracker recipes that were developed. In numerous aspects, including appearance (7.00 points), flavor (6.00 points), texture (7.13 points), taste (7.06 points), and overall satisfaction (7.23 points) ( $P < 0.05$ ), the Ca-fortified cracker recipe with 50% of Ca by RDA per serving scored significantly higher than other recipes. In terms of flavor, texture, and taste ( $P < 0.05$ ), the Ca-fortified cracker recipe with 25% of Ca by RDA per serving received the lowest satisfaction scores compared to the other recipes. Furthermore, the satisfaction score for both the Ca-fortified cracker recipe with 50% of Ca by RDA per serving and the control formula cracker was higher than 7.00, indicating that acceptable by participants (**Table 1**). Based on these results, the cracker formula containing 50% of the RDA for Ca was selected for the next phase of the study, which examined its physicochemical properties and effectiveness on bone health.

**Table 1.** Sensory evaluation scores on the cracker samples in different formulas.

Aspects	Control	Percent of Ca by RDA formula recipe		
		15	25	50
Appearance	7.10 $\pm$ 1.32 <sup>a</sup>	6.23 $\pm$ 1.69 <sup>b</sup>	6.16 $\pm$ 2.21 <sup>b</sup>	7.00 $\pm$ 1.48 <sup>a</sup>
Color	6.53 $\pm$ 1.88 <sup>a</sup>	6.40 $\pm$ 1.81 <sup>a</sup>	6.16 $\pm$ 2.10 <sup>a</sup>	6.33 $\pm$ 1.78 <sup>a</sup>
Flavor	6.46 $\pm$ 1.52 <sup>a</sup>	6.13 $\pm$ 1.45 <sup>a,b</sup>	5.90 $\pm$ 1.95 <sup>b</sup>	6.00 $\pm$ 1.61 <sup>a,b</sup>
Texture	5.83 $\pm$ 2.00 <sup>c</sup>	6.33 $\pm$ 2.18 <sup>b</sup>	5.86 $\pm$ 2.16 <sup>c</sup>	7.13 $\pm$ 1.61 <sup>a</sup>
Taste	7.13 $\pm$ 1.52 <sup>a</sup>	6.00 $\pm$ 1.98 <sup>b</sup>	5.76 $\pm$ 2.31 <sup>b</sup>	7.06 $\pm$ 1.79 <sup>a</sup>
Overall satisfaction	7.03 $\pm$ 1.44 <sup>a</sup>	6.50 $\pm$ 1.99 <sup>b</sup>	6.00 $\pm$ 2.10 <sup>c</sup>	7.23 $\pm$ 1.43 <sup>a</sup>

Total score = 9; Data presented as Mean $\pm$ SD; Different superscript letters in the same row were significantly different for given satisfaction aspect ( $P < 0.05$ ) 3.2 Physico-chemical and Ca content analysis.

Participants evaluated the control group's crackers and the recipe containing 50% of the RDA of calcium per serving as acceptable based on the findings of the sensory evaluation. It was chosen to carry out the intervention phase and physico-chemical investigations as a result. With 2.03 g/100 g for the control recipe and 2.42 g/100 g for the 50% of Ca by RDA per serving recipe, the ash

content of both recipes was similar. The 50% of Ca by RDA per serving recipe had 183.38 mg/100 g of Ca content, while the control recipe had 68.89 mg/100 g of the content. The 50% of Ca by RDA per serving recipe  $L^* = 42.24$ ,  $a^* = 6.33$ , and  $b^* = 21.55$ , as well as the control recipe  $L^* = 54.40$ ,  $a^* = 4.02$ , and  $b^* = 20.43$ , exhibited color analysis according to the Hunter Lab color Quest XE

technique. The 50% of Ca by RDA per serving recipe had a moisture level of 3.66 g/100 g, whereas the control recipe had a moisture value of 2.99 g/100 g. Moreover, **Table 2** shows that the protein level of the two recipes was comparable, being at 4.33 g/100 g for the control recipes and

4.46 g/100 g for the 50% of Ca by RDA per serving plan. The results showed that there were significant differences ( $P<0.05$ ) in all evaluated variables between the control recipe and the Ca-fortified recipe, except protein content (**Table 2**).

**Table 2.** Physico-chemical analysis of the chosen cracker formula.

Variable	Control recipe	50% Ca by RDA formula recipe
Ash (g/100 g)	2.03 ± 0.02 <sup>a</sup>	2.42 ± 0.03 <sup>b</sup>
Ca (mg/100 g)	68.89 ± 0.02	183.38 ± 0.01 <sup>b</sup>
Color		
L*	54.40 ± 0.02	42.24 ± 0.04 <sup>b</sup>
a*	4.02 ± 0.01	6.33 ± 0.02 <sup>b</sup>
b*	20.43 ± 0.05	21.55 ± 0.03 <sup>b</sup>
Moisture (g/100 g)	2.99 ± 0.10	3.66 ± 0.03 <sup>b</sup>
Protein (g/100 g)	4.33 ± 0.02	4.46 ± 0.02

<sup>a</sup>: Mean±SD; <sup>b</sup>: Significantly different with the control recipe at  $P<0.05$  compared by simple paired *t*-test

#### **Effectiveness of the selected Ca-fortified cracker formula on bone health parameters**

In the intervention phase, participants in the intervention group were assigned to consume 250 g of Ca-fortified crackers (containing 50% of RDA) for 12 weeks, while the control group maintained their routine dietary habits during the same period. At baseline, the results showed no significant differences in background characteristics, including

sex distribution, age, and education level between groups. Additionally, they showed no differences in lifestyle factors that potentially affect bone health. The length of weekly exercise for the control group was 69.00 minutes/week, and for the intervention group, it was 66.16 minutes/week. The length of daily sun exposure for the control group was 24.66 minutes/day, and for the intervention group, it was 30.50 minutes/day (**Table 3**).

**Table 3.** Background characteristics of participants.

Characteristics	Control (n=20)	Intervention (n=20)	P-value
Sex			
Male	10 (50) <sup>a</sup>	12 (60)	0.52 <sup>c</sup>
Female	10 (50)	8 (40)	
Education			
Bachelor's degree	18 (80)	17 (85)	0.63 <sup>c</sup>
Graduate degree	2 (20)	3 (15)	
Age (year)	29.50±7.63 <sup>b</sup>	28.10±7.18	0.55 <sup>d</sup>
Length of weekly exercise (minute)	69.00±67.43	66.16±67.05	0.88 <sup>d</sup>
Length of daily sun exposure (minute)	24.66±27.54	30.50±30.12	0.16 <sup>d</sup>

<sup>a</sup>: n (%); <sup>b</sup>: Mean±SD; <sup>c</sup>: Pearson's chi-squared test; <sup>d</sup>: Independent *t*-test.

Regarding effectiveness of the selected Ca-fortified cracker using cricket protein, results revealed no significant differences in bone health parameters and body composition between groups of participants at baseline, except for the BMD of

ankle bone. Participants in the intervention group (0.51 g/cm<sup>2</sup>) had significantly higher ankle BMD than the control group (0.47 g/cm<sup>2</sup>) ( $P<0.05$ ). At the endpoint (12<sup>th</sup> week), results indicated that the ALP of participants in the intervention group was

59.25 IU/l, which was significantly lower than the control group's 68.75 IU/l ( $P<0.05$ ). Additionally, both BMD values of wrist and ankle for participants in the intervention group (0.53 and 0.57 g/cm<sup>2</sup>, respectively) were significantly higher than those of participants in the control group (0.45 and 0.44 g/cm<sup>2</sup>, respectively) ( $P<0.05$ ). Lastly, the

body mineral weight at the endpoint for participants in the intervention group was 2.83 kg, which was significantly higher than the control group's 2.42 kg ( $P<0.05$ ). It also significantly increased compared to the baseline within the intervention group ( $P<0.05$ ) (Table 4).

**Table 4.** Bone health parameters outcome and body composition.

Parameters	Baseline		P-value <sup>b</sup>	Endpoint		P-value <sup>b</sup>
	Control (n=20)	Intervention (n=20)		Control (n=20)	Intervention (n=20)	
Body mass index (kg/m <sup>2</sup> )	22.40±3.39 <sup>a</sup>	22.55±3.03	0.88	22.85±2.97	22.80±2.82	0.95
alkaline phosphatase (IU/l)	64.75±17.85	71.40±17.44	0.24	68.75±17.18	59.25±10.38	<0.05
Body fluid (l)	29.27±2.98	29.73±2.49	0.60	29.13±3.06	29.57±2.37	0.61
Body muscle mass (kg)	19.43±2.31	19.87±2.19	0.54	19.37±2.05	19.66±1.73	0.62
Body fat mass (kg)	19.75±3.44	19.86±2.94	0.91	20.48±3.19	20.33±2.29	0.86
Minerals (kg)	2.49±0.17	2.50±0.26	0.87	2.42±0.16	2.83±0.17	<0.05
Resting metabolic rate (kcal)	1,304.8±127.5	1,339.2±132.2	0.40	1,317.4±138.8	1,333.2±105.4	0.68
T-score of wrist bone	-1.26±0.77	-1.11±0.76	0.54	-1.39±0.71	-1.02±0.82	0.13
BMD of wrist bone (g/cm <sup>2</sup> )	0.47±0.07	0.49±0.08	0.38	0.45±0.06 <sup>^</sup>	0.53±0.09	<0.05
T-score of ankle bone	-1.19±0.79	-1.12±0.78	0.78	-1.28±0.80 <sup>^</sup>	-1.05±0.84	0.37
BMD of ankle bone (g/cm <sup>2</sup> )	0.47±0.04	0.51±0.06	<0.05*	0.44±0.04 <sup>^</sup>	0.57±0.05	<0.05

<sup>a</sup>: Mean±SD; <sup>b</sup>: Independent t-test; Bone mineral density.

**Table 5.** Participants' dietary habits.

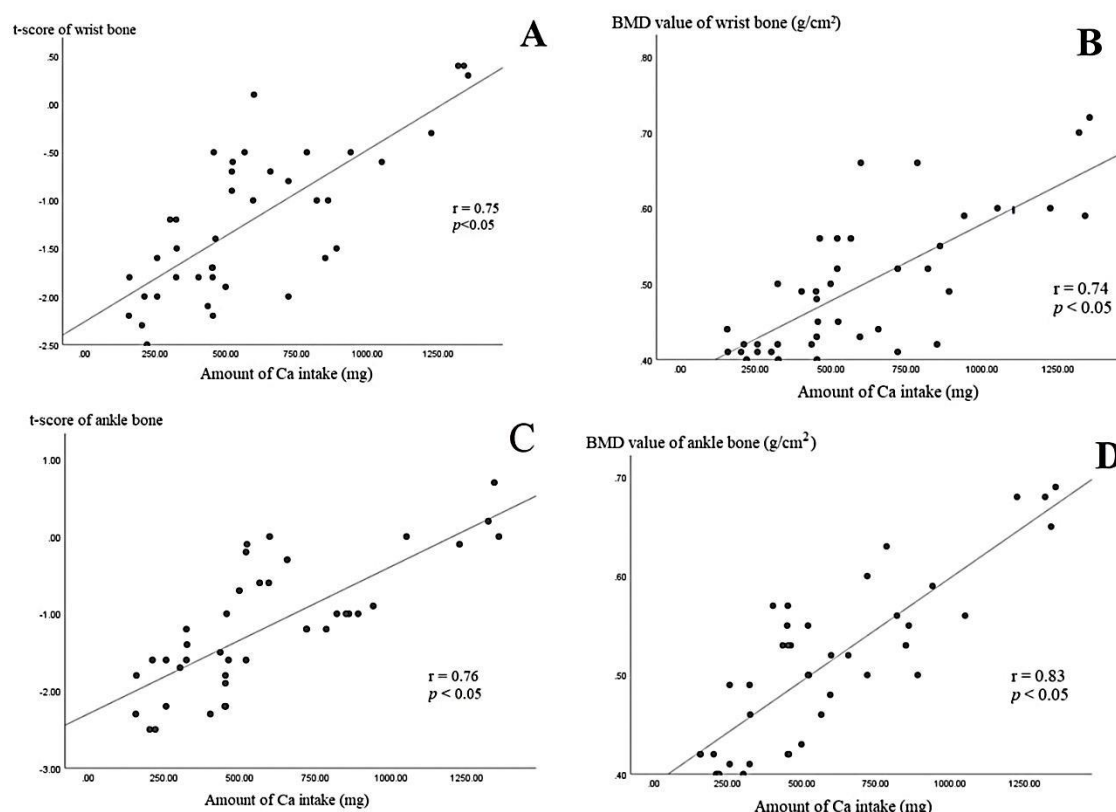
Nutrients intake	Control (n=20)	Intervention (n=20)	P-value <sup>b</sup>
Total kcal	1,825.8±261.200 <sup>a</sup>	1,816.1±195.000	0.89
Energy distribution			
%kcal from carbohydrate	56.40±6.03	58.55±8.02	0.34
%kcal from protein	12.25±4.55	14.05±6.02	0.29
%kcal from fat	31.35±3.93	27.40±5.00	<0.05
Ca	379.75±162.34	802.45±325.13	<0.05

<sup>a</sup>: Mean±SD; <sup>b</sup>: Independent t-test.

Regarding dietary habits among participants, results indicated that participants in the control group obtained a significantly higher percentage of kcal distribution from fat (31.35%) compared to those in the intervention group (27.40%,  $P<0.05$ ). Participants in the intervention group had a daily Ca intake of 802.45 mg, while the control group had a daily Ca intake of 379.75 mg. The results indicated a significant difference in daily Ca intake between the two groups ( $P<0.05$ , Table 5).

Moreover, when considering the correlation between the amount of Ca intake and bone density

parameters among all 40 participants in this study at the endpoint, the results showed significant positive correlations between Ca intake and both t-score and BMD values for wrist and ankle bones ( $P<0.05$ ). The correlation values between Ca intake and t-score and BMD value of the wrist bone were  $r=0.75$  and  $r=0.74$ , respectively (Figures 3A and 3B). Meanwhile, the correlations between Ca intake and t-score and BMD value of the ankle bone were  $r=0.76$  and  $r=0.83$ , respectively (Figures 3C and 3D).



**Figure 3.** Correlations between bone mass density parameters and Ca intake among participants (A; t-score of wrist bone, B; BMD value of wrist bone, C; t-score of ankle bone, D; BMD value of ankle bone).

## Discussion

The study's results showed that crackers made with 50% Ca by RDA formula recipe were rated similarly to the control recipe in terms of appearance satisfaction. However, the 15% and 25% Ca by RDA formula recipes received lower ratings compared to the other two. This difference might be attributed to the amount and flavor of calcium fortification, which was enhanced by the puffy texture of the crackers after frying due to calcium binding with carbohydrates (Chen *et al.*, 2016). Notably, all recipes received low satisfaction scores for color. This could be because the use of cricket protein powder, rather than more common protein sources like meat, fish, or shrimp, resulted in an unfamiliar darker color that participants found less appealing (Akullo *et al.*, 2023). The crackers made with the 50% Ca by RDA formula recipe achieved higher satisfaction scores for taste and texture compared to the other recipes. This superior performance can likely be

attributed to the increased calcium fortification in this formulation. The higher calcium content appears to have enhanced the stability of the crackers and improved their crunchiness, possibly due to a greater concentration of calcium molecules interacting with the carbohydrates. These improvements are evident in the tabulated ratings provided by the participants (Adi *et al.*, 2019). Interestingly, these findings differ from a previous study that examined noodles fortified with commercial calcium salts. In that case, the results found no significant effects on texture and springiness, suggesting that calcium fortification may impact various food products differently (Lin *et al.*, 2023).

The proximate analysis and color evaluation revealed differences in ash, moisture, and protein content between the control recipe and the Ca-fortified cracker recipe. These variations are likely due to the added Ca, which appears to have influenced the sample's overall composition.



Previous studies have indicated that higher Ca levels in foods can lead to increased ash content, greater moisture retention, and alterations in food color (Palacios *et al.*, 2021, Pawlos *et al.*, 2023). This prior finding may explain the observed differences in proximate composition and color between the two recipes. However, since the protein was added in equal amounts to both recipes, no significant difference was found in protein content between the control and Ca-fortified crackers.

The preliminary clinical effectiveness phase of the study found that, at the end of the study, the intervention group showed a higher percentage of body weight from mineral levels and greater BMD in the wrist and ankle bones compared to the control group.

This indicated the potential effectiveness of the developed Ca-fortified cracker using cricket protein in promoting bone mass density, as Ca is a crucial nutrient component of bones (Lin *et al.*, 2022). Adequate calcium intake therefore results in increased bone mass density. Previous study has also indicated that the amount of Ca received is correlated with increased bone mass density levels (Yao *et al.*, 2021). The dietary habits of participants in intervention group found that they had the intake of Ca 802 mg/day, while the control group was just 379 mg/day. This could be a potential factor affecting BMD outcomes, as prior studies have revealed that significant increases in bone mass can be observed when calcium intake exceeds 400 mg per day (Guo *et al.*, 2021). Regarding the ALP levels, which were lower in the intervention group compared to the control group, it is possible that sufficient calcium intake may help reduce bone resorption (Shu *et al.*, 2022), leading to these study results. However, the ALP levels of both groups are considered to be within the normal range, which should be between 20 to 140 IU/l (Sharma *et al.*, 2014).

Regarding dietary habits results, it was found that participants in the intervention group had a lower energy distribution from fat compared to the control group. It is possible that the educated participants attempted to reduce fat intake from

other sources when consuming the fried cracker (Lee *et al.*, 2021). When combined with the Ca received from the developed cracker, the intervention group's Ca intake reached 800 mg daily, which is close to the recommended daily Ca intake of 900 mg. Therefore, the Ca-fortified cracker using cricket protein can be considered an alternative product suitable for those who can drink milk normally, those who cannot digest lactose in milk, and those allergic to milk protein (Li *et al.*, 2023, Nayik *et al.*, 2021). This is because these groups need to avoid products and foods containing milk, which may affect their Ca intake. The development of this Ca-fortified cracker innovation thus provides an alternative source of dietary Ca. In addition, dietitians can recommend other Ca-rich food sources such as dark green vegetables, small fish, dried shrimp, hard tofu (Cormick and Belizán, 2019), or calcium supplements in various forms that the body can absorb well, such as Ca carbonate and Ca citrate (Shkempi and Huppertz, 2021). This should be coupled with appropriate sun exposure and exercise, which are two other factors that can help promote bone mass strength (Cheng *et al.*, 2022). The limitations of this research include the small population size studied over a short period, as well as the lack of measurement of other biochemical values indicating bone mass status, such as parathyroid hormone and vitamin D.

## Conclusion

In conclusion, the developed Ca-fortified Thai traditional cracker using cricket protein with obtained Ca 50% by RDA per portion (250 g) is acceptable and preliminarily effective to improve bone health.

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## Authors' contributions

Kosisuwan S, Sriwanich S, Aipirome S, and Aurareesuksakul A performed the data curation and investigation. Prapkree L gave supervision and proofread the manuscript. Singhato A conceptualized the study, write manuscript, and

were involved in funding.

### Conflict of interests

The authors declared no conflict of interests.

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### References

- Adi AC, Rachmah Q & Arimbi AN 2019. The acceptance and nutritional value of crispy noodles supplemented with moringa oleifera as a functional snack for children in a food insecure area. *Preventive nutrition and food science*. **24** (4): 387-392.
- Akkawi I & Zmerly H 2018. Osteoporosis: current concepts. *Joints*. **6** (2): 122-127.
- Akullo J, Kiage-Mokua B, Nakimbugwe D, Ng'ang'a J & Kinyuru J 2023. Color, pH, microbiological, and sensory quality of crickets (*Gryllus bimaculatus*) flour preserved with ginger and garlic extracts. *Food science & nutrition*. **11** (6): 2838-2851.
- Amphansap T, et al. 2021. Comparison of bone mineral density and vertebral fracture assessment in postmenopausal women with and without distal radius fractures. *Osteoporos sarcopenia*. **7** (4): 134-139.
- Assantachai P, Angkamat W, Pongpim P, Weattayasuthum C & Komoltri C 2006. Risk factors of osteoporosis in institutionalized older Thai people. *Osteoporosis international*. **17**: 1096-1102.
- Chen T-Y, Luo H-M, Hsu P-H & Sung W-C 2016. Effects of calcium supplements on the quality and acrylamide content of puffed shrimp chips. *Journal of food and drug analysis*. **24** (1): 164-172.
- Cheng J, Meng S, Lee J, Kwak H & Liu Y 2022. Effects of walking and sun exposure on bone density and balance in elderly with osteopenia. *Journal of bone and mineral metabolism*. **40** (3): 528-534.
- Cheng X & Zhao C 2023. The correlation between serum levels of alkaline phosphatase and bone mineral density in adults aged 20 to 59 years. *Medicine*. **102** (32): e34755.
- Clynes M, et al. 2020. The epidemiology of osteoporosis. *British medical bulletin*. **133** (1): 105-117.
- Cormick G & Belizán J 2019. Calcium Intake and Health. *Nutrients*. **11** (7).
- El-Hagrassy M, Duarte D, Thibaut A, Lucena M & Fregni F 2018. Principles of designing a clinical trial: optimizing chances of trial success. *Current behavioral neuroscience reports*. **5**: 143-152.
- Garg M & Mahalle N 2019. Calcium Supplementation: Why, Which, and How? *Indian journal of endocrinology and metabolism*. **23** (4): 387-390.
- Gold T, et al. 2019. Impact of fractures on quality of life in patients with osteoporosis: a US cross-sectional survey. *Journal of drug assessment*. **8** (1): 175-183.
- Guo X, et al. 2021. Association of dietary calcium intake with bone health and chronic diseases: Two prospective cohort studies in China. *Frontiers in nutrition*. **8**: 683918.
- Hanzlik R, Fowler S & Fisher D 2005. Relative bioavailability of calcium from calcium formate, calcium citrate, and calcium carbonate. *Journal of pharmacology and experimental therapeutics*. **313** (3): 1217-1222.
- Hermans W, et al. 2021. Insects are a viable protein source for human consumption: from insect protein digestion to postprandial muscle protein synthesis in vivo in humans: a double-blind randomized trial. *American journal of clinical nutrition*. **114** (3): 934-944.
- Hodges J, Cao S, Cladis D & Weaver C 2019. Lactose intolerance and bone health: The challenge of ensuring adequate calcium intake. *Nutrients*. **11** (4).
- Iki M 2012. Epidemiology of osteoporosis in Japan. *Clinical calcium*. **22** (6): 797-803.
- Kim K, Oh S & Hong Y 2018. Associations of serum calcium levels and dietary calcium intake with incident type 2 diabetes over 10 years: the Korean Genome and Epidemiology Study (KoGES). *Diabetology & metabolic syndrome*. **10**: 50.
- Kim T, Yong H, Kim Y, Kim H & Choi Y 2019. Edible insects as a protein source: A review of public perception, processing technology, and research trends. *Food science of animal resources*. **39** (4): 521-540.
- Lee J, et al. 2021. Association between nutrition education, dietary habits, and body image misperception in adolescents. *Asia Pacific journal of clinical nutrition*. **30** (3): 512-521.

- Li A, et al.** 2023. Advances in low-lactose/lactose-free dairy products and their production. *Foods*. **12** (13).
- Lin H, Chen G, Chang K, Bo Y & Sung W** 2023. Comparison of physicochemical properties of noodles fortified with commercial calcium salts versus calcium citrate from oyster shells. *Foods*. **12** (14): 2696.
- Lin S, Chen C, Cai X, Yang F & Fan Y** 2022. The concentrations of bone calcium, phosphorus and trace metal elements in elderly patients with intertrochanteric hip fractures. *Front endocrinol* **13**: 1005637.
- Maisont S, Samutsri W, Phae-Ngam W & Limsuwan P** 2021. Development and characterization of crackers substitution of wheat flour with jellyfish. *Frontiers in nutrition*. **8**: 772220.
- Murugu D, et al.** 2021. From farm to fork: crickets as alternative source of protein, minerals, and vitamins. *Frontiers in nutrition*. **8**: 704002.
- Nayik G, et al.** 2021. Recent insights into processing approaches and potential health benefits of goat milk and its products: A review. *Frontiers in nutrition*. **8**: 789117.
- Palacios C, et al.** 2021. Calcium-fortified foods in public health programs: considerations for implementation. *Annals of the New York Academy of Sciences*. **1485** (1): 3-21.
- Pawlos M, Znamirska-Piotrowska A, Kowalczyk M, Zagula G & Szajnar K** 2023. Possibility of using different calcium compounds for the manufacture of fresh acid rennet cheese from goat's milk. *Foods*. **12** (19).
- Pongchaiyakul C, Charoenkiatkul S, Kosulwat V, Rojroongwasinkul N & Rajatanavin R** 2008. Dietary calcium intake among rural Thais in Northeastern Thailand. *Journal of the medical association of Thailand*. **91** (2): 153-158.
- Pouresmaeili F, Kamalidehghan B, Kamarehei M & Goh Y** 2018. A comprehensive overview on osteoporosis and its risk factors. *Therapeutics and clinical risk management*. **14**: 2029-2049.
- Sharma U, Pal D & Prasad R** 2014. Alkaline phosphatase: an overview. *Indian journal of clinical biochemistry*. **29** (3): 269-278.
- Shkempi B & Huppertz T** 2021. Calcium absorption from food products: food matrix effects. *Nutrients*. **14** (1).
- Shu J, Tan A, Li Y, Huang H & Yang J** 2022. The correlation between serum total alkaline phosphatase and bone mineral density in young adults. *BMC Musculoskelet Disord*. **23** (1): 467.
- Skrivervik E** 2020. Insects' contribution to the bioeconomy and the reduction of food waste. *Heliyon*. **6** (5): e03934.
- Stull V, et al.** 2018. Impact of edible cricket consumption on gut microbiota in healthy adults, a double-blind, randomized crossover Trial. *Scientific reports*. **8** (1): 10762.
- Sun J-y, et al.** 2021. Impact of serum calcium levels on total body bone mineral density: A mendelian randomization study in five age strata. *Clinical nutrition*. **40** (5): 2726-2733.
- Tai V, Leung W, Grey A, Reid I & Bolland M** 2015. Calcium intake and bone mineral density: systematic review and meta-analysis. *British medical journal*. **351**: h4183.
- Wichchukit S & O'Mahony M** 2015. The 9-point hedonic scale and hedonic ranking in food science: some reappraisals and alternatives. *Journal of the science of food and agriculture*. **95** (11): 2167-2178.
- Yao X, Hu J, Kong X & Zhu Z** 2021. Association between dietary calcium intake and bone mineral density in older adults. *Ecology of food and nutrition*. **60** (1): 89-100.