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EDITORIAL TEAM

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Welcome message from Editor-in-Chief

Dear authors, reviewers, and readers

We are honored to present the first issue of the fifth volume of the Journal of Science and Agricultural Technology (JSAT), the official journal of the Faculty of Science and Agricultural Technology, Rajamangala University of Technology Lanna (RMUTL), Thailand. This issue includes five research articles from various institutions that contributed to this issue. The JSAT has been published in Thai Journal Online (ThaiJO), indexed in Google Scholar, Thai Citation Index (TCI), and Digital Object Identifier (DOI) under the National Research Council of Thailand. The journal will publish high-quality articles under an intense peer-review process with solid support from various educational institutions domestically and abroad.

As an Editor-in-Chief, I am so grateful for the support from our submitting authors, reviewers, and staff. I promise to move forward to gain international recognition, preparing for a higher index ranking. In addition, I strongly encourage researchers around the globe to submit manuscripts to share knowledge and promote the growing field of science and agricultural technology.

Best regards,

Assoc. Prof. Dr. Suntorn Wittayakun

Editor-in-Chief Journal of Science and Agricultural Technology
Faculty of Science and Agricultural Technology
Rajamangala University of Technology Lanna, Thailand.

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ABOUT THE JOURNAL

Journal of Science and Agricultural Technology (JSAT) publishes original research contributions covering science and agricultural technology such as:

• Natural and applied sciences: biology, chemistry, computer science, physics, material science and related fields. Papers in mathematics and statistics are also welcomed, but should be of an applied nature rather than purely theoretical.

• Agricultural technology: plant science, animal science, aquatic science, food science, biotechnology, applied microbiology, agricultural machinery, agricultural engineering and related fields.

Furthermore, the JSAT journal aims to span the whole range of researches from local to global application.

The JSAT is published two issues a year.
Issue 1: January - June
Issue 2: July - December

Submissions are welcomed from international and Thai institutions. All submissions must be original research not previously published or simultaneously submitted for publication or submitted to other journals. Manuscripts are peer reviewed using the double-blinded review system by at least 3 reviewers before acceptance. There is no publication or processing fee.

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Editorial office:

Faculty of Science and Agricultural Technology,
Rajamangala University of Technology Lanna,
128 Huaykaew Rd., Changphuek, Muang, Chiang Mai, 50300 Thailand.
Tel/Fax: +665392 1444 Ext. 1506

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CONTENTS

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Detecting the antifungal activity of the piper genus against *Colletotrichum capsici*, the cause of chili anthracnose

Sukee Sukdee[1*], Satra Ladpala[1], Piboon Chayaowsakoon[1]

[1]Faculty of Science, Lampang Rajabhat University, Lampang 52100, Thailand

***Corresponding author**: sukee_sukdee@hotmail.com*

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ABSTRACT

This study investigated the antifungal activities of plant extracts that inhibit *Colletotrichum capsici*. Four species of the Piper genus were assessed for their potential as effective natural antifungal agents against chili pathogens. Plant extracts were tested *in vitro* at concentrations of 5, 10, and 20 mg/mL using the poisonous food diffusion technique; they effectively inhibited mycelia growth of *Colletotrichum capsici*, significantly affecting fungal toxicity. The *Piper betle* L. crude extracts had the highest effectiveness (> 90%) against fungicides. The antifungal properties, like the *Piper retrofractum* Vahl., showed a significant inhibitory percentage (> 80%). Furthermore, hexane and ethyl acetate extracts of *Piper nigrum* L. and *Piper sarmentosum* Roxb. demonstrated excellent antifungal activity (> 80%). The phytochemical investigation of sample extracts revealed flavonoids, phenols, alkaloids, terpenoids, and steroids. As a result, the biological activity of plant extracts inhibited the growth of *Colletotrichum capsici* mycelia; they could serve as a source for the development of eco-friendly organic fungicides.

Keywords: antifungal, *Colletotrichum capsici*, chili, anthracnose, piper genus

INTRODUCTION

In the tropics, chili (*Capsicum annuum* L.) is considered an essential crop (May and Sang, 2016). Approximately 3.74 million hectares and 3.84 million tons of chili were cultivated on a global scale (FAO., 2020; Nicephore et al., 2021). Meanwhile, the harvest area in Thailand is approximately 5.50 thousand hectares with a yield of 2.84 million tons; the estimated revenue is $426.67 million (Chatsuda et al., 2021). However, causal fungal infections caused by *Colletotrichum* sp. pose the greatest threat to chili production from anthracnose disease (Raghavendra et al., 2020). Therefore, the infected chili was anthracnose, which caused worldwide losses of up to 50% (Po et al., 2008). This effect is responsible for both pre- and post-harvest chili damage. Fifty percent of cause losses have been reported in Malaysia (Wong et al., 2020), 10–54% in India, 20–80% in Vietnam (Raj et al., 2020), and up to 80% in Thailand (Manju et al., 2020). Six species of *Colletotrichum*, including *Colletotrichum capsici, C. siamense, C. acutatum, C. scovillei, C. asianum,* and *C. gloeoporioides*, have been linked to anthracnose in Indonesia, India, Korea, and Thailand (Than et al., 2008; May and Sang et al., 2016; Chatsuda et al., 2021). Chemicals, biological agents, or plant extracts could be used to control anthracnose disease (Manju et al., 2020). Chemicals have been used to prevent the disease anthracnose; the fungicide control was simple to manipulate and quick to respond. In contrast, long-term use of pathogenesis fungicide chemicals will result in human and environmental toxicity risks. Another option is natural plant products, which are essential sources of fungicide and are nontoxic to animals and the environment; these are regarded as environmentally friendly with excellent care. According to research, plant extracts and bio-control agents can combat anthracnose diseases in chili, such as inhibiting fungal growth with a 3% garlic bulb extract. *Acorus calamus* L., *Cymbopogon martinii* oil, and *Azadirachta indica* oil plant extracts were effective in inhibiting the growth of the anthracnose fungus (Po et al., 2008; May and Sang, 2016); *Azadirachta indica*, *Swietenia mahagoni*, and *Allium sativum* were the combination plant extracts that had a positive effect on disease reduction and crop yield in chili (Raj et al., 2020; Manju et al., 2020).

Piper retrofractum Vahl has literature on traditional medicines derived from various plant parts that treat anti-flatulent, antitussive, antioxidant, expectorant, antibacterial, anti-inflammatory, antimicrobial, and antifungal activities (Wan et al., 2020). This plant was evaluated for its efficacy against bacterial pathogens, including *Streptomyces albus, Salmonella typhi, Pseudomonas aeruginosa, Escherichia coli,* and *Bacillus megaterium*, as well as the fungus *Aspergillus niger* (Mohib and Mustafa, 2007). In addition to exhibiting excellent

antibacterial activity, the extracts also demonstrated antifungal activity. Similarly, the bioactive methanol extract of *Piper retrofractum* Vahl was effective against pathogenic fungi, including *Fusarium moniliforme*, *F. oxysporum* DOAC2269, *Colletotrichum gloeosporioides* DOAC2213, and *C. acutatum* DOAC2285; the crude extracts demonstrated the potential for broad-spectrum antifungal activity in plant pathogens (Wattana, 2017). In addition, piperine showed significant antifungal activity against *Staphylococcus aureus* and *Bacillus subtilis* with MIC values of 225 g/mL (Wan et al., 2020). Lignans, sterol, alkaloids, flavones, tannin, and phenol were identified as antifungal constituents of *Piper retrofractum* Vahl.'s phytochemical constituents (Wan et al., 2020).

Piper nigrum L. contains an active component with diverse pharmacological properties, including antitumor, antioxidant, anti-inflammatory, antibacterial, insecticidal, and antifungal properties, among others (Nisar et al., 2012; Zoheir and Aftab, 2014). The antibacterial activity of piperine extract was demonstrated against *P. putida* and *Staphylococcus aureus*, with the highest inhibition observed in *Staphylococcus* (Krishna et al., 2019). Leaf extract with methanol has shown antimicrobial activity against *E. coli*, and leaf extract with ethanol is highly active against *S. aureus*; these extracts protect against dangerous pathogenic microorganisms (Mohd et al., 2014). The investigation of biologically significant phytochemicals, such as phenolics, flavonoids, alkaloids, terpenes, lignans, and steroids, could yield antifungal (Winda et al., 2021).

Piper betle L. was used to treat diseases in traditional medicine. This plant is believed to have bioactivity, including immunomodulatory, antifilarial, antileishmanial, antiamoebic, antioxidant, anti-inflammatory, antimicrobial, and antifungal properties (Biswajit et al., 2016). According to the research, there was a high activity level against the pathogens tested. For instance, *Colletotrichum capsici, Fusarium pallidoroseum*, *Botryodiplodia theobromae*, *Alternaria alternate*, *Penicillium citrinum*, *Phomopsis caricae-papayae*, and *A. niger* were inhibited significantly; the most minor inhibitory concentrations of ethanol extracts against these plant pathogens ranged from 0.01 mg/mL to 1 mg/mL (Kushagra et al., 2011). *Colletotrichum capsici* radial growth was most inhibited by leaf extract at a concentration of 10 µg/mL; methanol, chloroform, and acetone extracts inhibited growth by 85.25%, 78.53%, and 73.58%, respectively (Lucy et al., 2011). Researchers demonstrated the *in vitro* antifungal activity of hydroxychavicol isolated from *P. betle* L. against 124 fungal strains, including *Aspergillus* species (e.g., *A. flavus*, *A. parasiticus*, *A. niger*, and *A. fimigatus*) (Intzar et al., 2010). Tannins, essential oils, terpenoids, alkaloids, steroids, and phenol were among the phytochemical constituents extracted from *P. betle* L.; these bio-compounds could reduce the severity of fungus disease (Depi et al., 2020).

The pharmacological properties of *Piper sarmentosum* include anti-inflammatory, antioxidant, antimalarial, antimicrobial, antiprotozoal, antimicrobial, and antifungal properties (Rahman et al., 2016; Azelan et al., 2020). The IC_{50} values of the methanol extracts of *P. sarmentosum* against the fungi *P. fuscovaginae* and *Xanthomonas oryzae* were 10.42 and 24.69, respectively (Rahman et al., 2014). In addition, the essential oil exhibited high antifungal activity against *Rhizoctonia solani* and *Bipolarisoryzae* (Pragatsawat and Warinthorn, 2017). Moreover, at a concentration of 100 mg/mL, the aqueous extract inhibited the growth of *Fusarium verticillioides* with a diameter of 7.3 mm (Maizatul and Aiesyaa, 2020). *Piper sarmentosum* contains phytochemicals such as flavonoids, phenolic, saponins, terpenoids, steroids, tannins, and alkaloids; consequently, plant extracts are effective against fungi (Atefeh et al., 2013).

This study aimed to evaluate the efficacy of plant extracts in inhibiting *Colletotrichum capsici*, the agent responsible for chili anthracnose.

MATERIALS AND METHODS

Plant extracts

The experiment focuses on Piper genus plants collected in Thailand's Lampang province, including *Piper retrofractum* Vahl, *Piper nigrum* L., *Piper betle* L., and *Piper sarmentosum* Roxb. Plants were identified by the Biology Laboratory of Lampang Rajabhat University's Faculty of Science. The leaves were air-dried at room temperature, ground into a fine powder, and then successively percolated with n-hexane, ethyl acetate, and methanol (50 g × 0.3 L × 3 days three times) at room temperature, respectively. The filtrate solutions were evaporated under low pressure at 40 °C, and the resulting crude extracts were used to test bioactivity.

Phytochemical screening

The screenings of plant extracts utilize the method described by Trease and Evans (1989); testing methods reveal plants' chemical composition, such as phenols, flavonoids, alkaloids, terpenoids, and steroids (Rao et al., 2016).

Colletotrichum capsici material

Samples infect anthracnose disease with *Colletotrichum capsici* on chili fruit brought from regions Lampang province. The method used for separating and purifying fungi employs Tun et al.'s procedure (Tun et al., 2018).

Molecular variability

The identification of *Colletotrichum capsici* was based on a molecular analysis of DNA; using a modified version of Doyle and Doyle's Cetyl Trimethyl Ammonium Bromide technique, they were isolated from each pure culture. Under the following thermal settings, the ITS of rDNA was amplified by ITS3 (5′GCATCGATGAAGAACGCAGC3′) and ITS4 (5′TCCTCC GCTTATTGATATGC3′): 95 ºC for 5 min, 30 cycles of 95 ºC for 30 s, 60 ºC on 30 s, and 72 ºC on 1 min. During PCR purification, the Gel/PCR DNA Fragments Extraction Kit (Geneaid), Taiwan, was utilized. The Macrogen, Inc. genetic analyzer was used to determine the sequences. Using the Basic Local Alignment Search Tool, sequences were compared to other sequences in the Gen Bank database (BLAST) (Meghana and Hiremath, 2019).

Evaluation of plant extracts inhibition Colletotrichum capsici in vitro

Evaluation of the efficacy of plant extracts against *Colletotrichum capsici* using the poisoned food diffusion technique. Four crude plant extracts were dissolved in lukewarm PDA and thoroughly mixed to produce a final 5, 10, and 20 mg/mL concentration. A procedure developed by Tun et al. (2018) was used to assess the antifungal activity of plant extracts (Tun et al., 2018). Fungi toxicity was reported as a percentage of inhibition and calculated according to the equation to be compared with the control.

$$P = 100 - \frac{100 \times R^2}{C^2}$$

P = Percentage of inhibitory activity against radial growth

C^2 = Radial growth of the fungal in the control

R^2 = Radial growth of the fungal in the treatment

Statistical analysis

All experiments were repeated five times. In all cases, analysis of variance (ANOVA) indicated that the data between the five repetitions were similar ($P > 0.05$). Thus, data of all variables from all five experiments were combined. Anthracnose control data obtained in all experiments of the extract concentration were used to estimate the effective extract concentration (%) to reduce colony diameter for each plant extract by two-way analysis of variance analysis, with the extract concentration as the independent variable and anthracnose as the dependent variable. The ANOVA (two-factor without replication) was used to compare all treatments' mean percentage of mycelial inhibition. The differences at $\alpha = 0.01$ were significant.

RESULTS AND DISCUSSION

Phytochemical screenings are shown in Table 1, including flavonoids, phenols, alkaloids, terpenoids, and steroids. Details the phytochemical evaluations of various plant extracts dissolved in solvents such as n-hexane, ethyl acetate, and methanol. Four plants were collected from a native plant for this experiment. This was research on using antifungal plant extract to treat chili diseases, except for *Piper nigrum* L. and *Piper sarmentosum* Roxb, which could not detect flavonoid compounds. Qualitative analyses of phytochemicals revealed the presence of steroids, alkaloids, and terpenoids in all plant extracts.

Table 1. Phytochemical screenings of plants

Plants	Flavonoid			Phenolic			Alkaloid			Terpenoid			Steroid		
	H	**E**	**M**	**H**	**E**	**M**	**H**	**E**	**M**	**H**	**E**	**M**	**H**	**E**	**M**
P. retrofractum	−	+	−	+	−	−	+	−	+	+	+	+	+	+	+
P. nigrum L.	−	−	−	−	−	+	+	+	+	+	+	+	−	+	+
P. betle L.	−	−	+	+	−	+	+	+	+	+	+	+	+	+	+
P. sarmentosum	−	−	−	+	+	+	+	+	+	−	−	+	+	+	+

H: n-hexane extracts, **E**: ethyl acetate extracts, **M**: methanol extracts, +: detected, −: non-detected

The plant extract exhibited a positive effect against the fungus *Colletotrichum capsici*, as indicated by the percentage inhibitions listed in Table 2. To advance potential new fungicides, it is necessary to investigate in vitro antifungal activity. Experiments with various solvents revealed that plant leaf extracts significantly ($P < 0.01$) inhibit mycelia growth. When the concentrations were 5, 10, and 20 mg/ml, respectively, the restraining was significantly enhanced (Fig. 1). The **H** extracts of *P. betle* L. demonstrated the greatest mycelia growth inhibition (95.76%). In the **H** extracts of *P. retroctum, P. nigrum* L., and *P. sarmentosum*, the percentage of mycelia growth inhibition efficacy was found to be 89.43, 85.75, and 76.15, respectively. Experiments with **E** extracts of *P. betle* L., *P. nigrum* L., *P. retrofractum*, and *P. sarmentosum* revealed that a concentration of 20 mg/mL significantly inhibited the pathogen by 95.36, 93.11, 90.66, and 87.38%, respectively. The **M** plant extracts inhibited the growth of *Colletotrichum capsici* effectively. They found that a concentration of 20 mg/mL inhibited the mycelia growth of *P. retrofractum, P. betle, P. Sarmentosum,* and *P. nigrum* by 95.57, 94.21, 68.05, and 2.47%, respectively.

Table 2. Inhibition of *Colletotrichum capsici* with plant extracts

Percentage of inhibition of *Colletotrichum capsici* with plant extracts										
Plant	**Con.** (mg/ml)	**H** MR ± SD (mm)		%	**E** MR ± SD (mm)		%	**M** MR ± SD (mm)		%
Control		50.69	±0.05	0	51.26	±0.47	0	48.51	±0.31	0
Pn	5	23.08	±1.00	79.28	19.63	±0.28	85.33	56.58	±0.44	No
	10	21.57	±0.27	81.89	16.76	±0.09	89.3	53.27	±0.16	No
	20	19.14	±0.34	85.75	13.45	±0.06	93.11	47.91	±0.61	2.47
Pr	5	21.83	±0.39	81.45	22.98	±0.09	79.9	14.31	±0.53	91.3
	10	19.14	±0.13	85.75	19.15	±0.57	86.04	12.24	±0.23	93.63
	20	16.48	±0.14	89.43	15.67	±0.07	90.66	10.21	±0.13	95.57
Ps	5	42.84	±0.14	28.57	31.66	±0.21	62.1	39.32	±0.08	34.29
	10	34.57	±0.48	53.48	27.07	±0.47	72.11	36.47	±0.12	43.48
	20	24.76	±0.75	76.15	18.21	±0.23	87.38	27.42	±0.46	68.05
Pb	5	13.65	±0.17	92.75	16.79	±0.57	89.27	28.29	±0.35	66.00
	10	11.1	±0.13	95.2	13.87	±0.50	92.68	20.71	±0.53	81.77
	20	10.44	±0.09	95.76	11.04	±0.26	95.36	11.67	±0.24	94.21

Each value represented the mean radial growth (MR) (5 replicates) ± standard deviation (SD); Significant differences ($P < 0.01$) are indicated by different alphabets. No = no inhibition; **Con**.: Concentration; **Pn**: *P. nigrum* L.; **Pr**: *Piper retrofractum* Vahl.; **Ps**: *P. Sarmentosum*; **Pb**: *P. betle* L.

This study revealed that crude leaf extracts from all four piper plants inhibited the growth of *Colletotrichum capsici* mycelia in a manner consistent with antifungal activity. Compared to previous research, the percentage of inhibition by crude plant extract of the Piper genus at 20 mg/mL was significantly higher when compared to the positive control of *Colletotrichum capsici.* According to research, extracts of the Piper genus demonstrated antibacterial and antifungal activities. Several flavonoids, phenols, alkaloids, terpenoids, and steroids were previously reported as phytochemicals from the Piper genus. This will be elaborated upon in the following section.

The number of active chemicals in an extract that can inhibit the growth of pathogens is shown to be inversely correlated with its concentration; specifically, the higher the extract concentration, the lower its ability to promote the growth of *Colletotrichum capsici*. Figure 1 shows a significant effect on the extracts tested. Extraction and concentration are greatly influential in inhibiting the growth of the pathogen *Colletotrichum capsici*. The higher the concentration of the extract, the higher the percentage of inhibition, and the growth of mycelium on PDA media decreases to an inhibition zone which feat *Colletotrichum capsici* pathogens hard to grow due to exposure to active fungicides in the extract. The leaf of *P. betle* L. has anti-fungal properties with essential oil components, namely eugenol, acetyl eugenol, and hydroxychavicol compounds (Intzar et al., 2010) that can inhibit pathogen growth (Kushagra et al., 2011; Depi et al., 2020). *Piper retrofractum* Vahl., showed

demonstrated antifungal activity. Similarly, the bioactive extract of *P. nigrum* L. (Krishna et al., 2019) and *P. sarmentosum* (Maizatul and Aiesyaa, 2020) were effective in inhibiting anthracnose (Wattana, 2017). Extracts have antifungal properties because they are found to contain active components; piperine, β-caryophyllen, limonine, eugenol, acetyl eugenol, and hydrocinnamic acid compounds, consequently could effectively inhibit the growth of fungal mycelium (Atefeh et al., 2013; Wan et al., 2020; Winda et al., 2021). The test results are reasonably compatible with earlier research reports for all experimental plants. Therefore, the crude extracts have the potential for antifungal activity in plant pathogens.

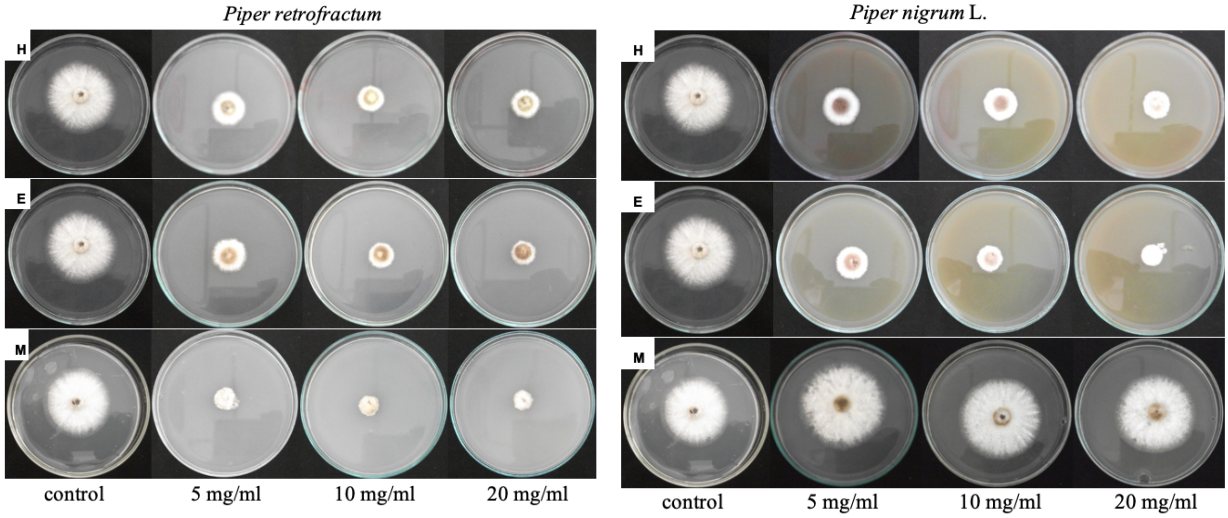

Figure 1. Inhibition of *Colletotrichum capsici* with *Piper* genus (in PDA).

CONCLUSIONS

This study demonstrates the highest effectiveness against the chili-causing fungus *Colletotrichum capsici*. Extracted crude plants revealed chemical components, including flavonoids, phenols, alkaloids, terpenoids, and steroids. Utilizing natural product plants as fungicides in organic agriculture has the potential to benefit from the results of this study. This study revealed that bioactive metabolites from plants have the highest potential as fungicides for agriculture and will contribute to the future management of these essential diseases.

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Effect of some fermentation factors on the fermented tiger stripe peanut production by *Rhizopus microsporus* fungus

Sompohn Srakaew[1], Panarin Preechawattanakorn[1], Piyanuch Niamsup[1], Chutamas Maneewong[1], Suphat Phongthai[2], Tomohiro Ikeda[3], Shin Yasuda[3] and Pairote Wongputtisin[1*]

[1]Program in Biotechnology, Faculty of Science, Maejo University, Chiang Mai 50290, Thailand
[2]Faculty of Agro-Industry, Chiang Mai University, Chiang Mai 50100, Thailand
[3]School of Agriculture, Tokai University, Kumamoto 861-2205, Japan

***Corresponding author**: pairotewong@gmail.com*

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ABSTRACT

Tiger stripe peanut (*Arachis hypogaea* L.) (TSP) is a famous Thailand geographical indication (GI) product of the Mae Hong Son province, Thailand. However, the value-added products of this peanut have been required by many local entrepreneurs. Therefore, the production of fermented TSP, a Tempe-like product, by *Rhizopus microsporus* fungus was proposed in our research. The objectives of this study were to investigate the effects of inoculum size (10^5, 10^6, and 10^7 spores/g), incubation temperature (25, 30, 35, and 40°C), and fermentation time (1–7 days) on the soluble reducing sugar, available phosphate and soluble protein of fermented TSP. Subsequently, some chemical properties of the product were studied. The results showed optimal fermentation conditions included an inoculation size of 10^7 spores/g and incubation at 35°C for two days. Moreover, the results from the proximate analysis study demonstrated that the crude protein content of fermented TSP increased by approximately 17% (by DW). The fat, carbohydrate, and crude fiber contents decreased by 3.5, 5.9, and 4.2% (by DW) compared to unfermented TSP, while their calculated energy contents were between 538-539 kcal/100 g DM. Total phenolic content (gallic acid equivalent, mg/g DW) and ABTS (2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid)) scavenging antioxidant capacity (Trolox equivalent, mg/g DW) of TSP were enhanced by approximately 2.6 and 1.9 folds, respectively, after fungal fermentation. Overall, the fermented TSP obtained from these optimized processes will be a new nutritious food product. However, further study on its health benefits will be able to apply this product as a novel functional Thai food.

Keywords: tiger stripe peanut, process optimization, functional food, fermentation

INTRODUCTION

Tiger stripe peanut (*Arachis hypogaea* L.) (TSP) or “Kalasin 2” and “Thua-Lai-Suer” in Thai is a local peanut Cultivar that was collected from the International Crops Research Institute for the Semi-Arid Tropics, India, in 1973. It had been selectively bred by the Kalasin Agricultural Research and Development Center since 1979. Nowadays, it is widely cultivated by farmers in several regions of Thailand. However, it is suitably cultivated in 4 districts of Mae Hong Son province in northern Thailand, i.e., Muang, Pang Ma Pa, Kun Yuam, and Pai, because of appropriate weather and soil conditions. The quality of TSP grown in Mae Hong Son is outstanding and is domestically accepted among consumers because it tastes sweet and creamy. Moreover, the texture is crunchy. After TSP and its relevant processing products became well known, a policy and guideline for quality control (including internal control and traceability) were issued by the Mae Hong Son Provincial Administrative Organization. This promotion policy led to the registration of the Mae Hong Son TSP as the Thailand geographical indication (GI) product in 2019 by the Department of Intellectual Property, Thailand. The GI sign can guarantee a specific geographical origin, quality, and reputation of products. Typically, peanuts are a source of oil, proteins, carbohydrates, and minerals. Arya et al. (2016) reported the data according to the USDA National Nutrient database that protein, carbohydrate, and fat content is about 25.8, 16.13 and 49.24% (by wt.), and they are also excellent sources of active compounds, such as resveratrol, phenolic acids, flavonoids, and phytosterols. Moreover, peanuts contain high unsaturated fatty acids, low saturated fatty acids, and cholesterol-free (Arya et al., 2016). Thus, the consumption of peanuts is expected to improve serum lipid profiles, decrease LDL oxidation, cardio-protective effect, and the risk reduction of cardiovascular disease, type 2 diabetes,

and cancers (Arya et al., 2016; Çiftçi and Suna, 2022).

In Thailand, many community enterprises and local small and medium enterprises (SMEs) commercially produce and process the TSP and relevant products. However, the variety of products is still low. A salted roasted TSP seems to be the leading and well-known product in the market. The research and development of novel products, especially functional foods, from TSP to consumers are of interest and required among those enterprises. In this study, we focused on the product development of TSP by using microbial fermentation technology, an effective and simple technique, and a low-cost process to enhance the nutritional quality and value of such agricultural raw materials. Fermentation allows microorganisms to make several biochemical changes in the substrates, improving the nutritional value, degrading anti-nutritional factors, and increasing nutrient bioavailability and bioactivities. (Mukherjee et al., 2016; Lo et al., 2022). Furthermore, the market demand for healthy foods, especially plant-based protein foods for vegetarians and vegans, has expanded. In this study, solid-stage fermentation by *Rhizopus microsporus* fungus was proposed. There are six varieties of *R. microsporus*, i.e. vars *microsporus*, *azygosporus*, *chinensis*, *oligosporus*, *rhizopodiformis* and *tuberosus*, based on their spore size and shape (Dolatabadi et al., 2013). This fungus has been used to produce oriental fermented foods for centuries. It is the predominant fermenting microbe found in Tempe, a local fermented soybean food of Indonesia, especially current Tempe products. The commercial Tempe starter has been widely used instead of natural starter (Sjamsuridzal et al., 2021). Therefore, the fermented TSP product was proposed and developed in our study to serve these recent demands. The objectives of this study were to study the effects of an inoculum size, incubation temperature, and fermentation time on the fermentation efficiency of TSP and to evaluate some nutritional quality, i.e., proximate analysis, phenolic content, and antioxidant activity, of the obtained product.

MATERIALS AND METHODS

Raw material

Fresh seeds of tiger stripe peanut (TSP) were collected from a product cultivated by the community enterprise in Pang Ma Pa district, Mae Hong Son province. Seeds were cleaned and de-hulled before being stored in a vacuum-sealed plastic bag at below 10°C.

Fungus

Rhizopus microsporus BG5 from the Agro-Industrial Biotechnology Laboratory, Faculty of Science, Maejo University, was used in this study. The fungal starter was prepared as a spore suspension in 0.85% (w/v) NaCl solution after being cultivated on potato dextrose agar (PDA) at 30°C. The spores were enumerated by counting them in a hemacytometer.

Effect of fermentation factors on the quality of fermented TSP

The dehulled TSP was soaked in distilled water containing 0.5% (w/v) lactic acid (KemAus™, Australia) for 12 h at 4°C, afterward drained, steamed until cooked, and cooled down at room temperature. Cooked TSP was used as the raw material in the production of fermented TSP. The fermentation was conducted in the 7x10 cm polyethylene Ziplock bag (without perforation) with 50 g of TSP (dry weight) and generally fermented with 10^6 spores/g, 30°C for 24 h. The optimal conditions to ferment TSP were investigated in this experiment according to the one-variable-at-a-time method. Three important factors involving the *Rhizopus* fermentation were optimized, i.e., inoculum size (10^5, 10^6, and 10^7 spores/g), incubation temperature (25, 30, 35, and 40°C), and fermentation time (1 to 7 days). Subsequently, the fermentation of all treatments was terminated by air drying at 70°C until constant weight and grinding into fine powders using a grinder machine (ARTC, UAE). The powders were stored in a sealed polypropylene bag at −20°C for further analysis. The content of soluble reducing sugar, protein, and phosphate of fermented TSP were the main criteria for consideration. Moreover, the finished product and raw material were subjected to analysis of their contents of macronutrients (proximate analysis), total phenolic content, and ABTS radical scavenging antioxidant capacity.

Chemical analysis of some fermented TSP nutrition

One gram of fermented TSP powder was mixed well with 9 ml DI water, sonicated without temperature control for 30 min, and centrifuged to collect the supernatant (water-soluble fraction) at 10,000 RPM and 10 min for the analysis of soluble reducing sugar, protein, and phosphate contents. To determine the soluble reducing sugar content, DNS (3,5-dinitrosalicylic acid method modified from the protocol of Miller (1959), was performed (Wongputtisin et al., 2015). The reducing sugar content was calculated from the calibration curve of standard D-glucose (Ajax-Finechem, Australia)

plotted versus the light absorbance at 540 nm, and the results were expressed as mg/ g DW. The soluble protein content was analyzed according to the spectrophotometry method described by Bradford (1976). The Coomassie Brilliant Blue G-250 - protein complex was measured by the light absorbance at 595 nm and estimated protein content by the standard curve of bovine serum albumin (BSA) (HiMedia, India). The soluble phosphate or available phosphate content was determined according to the principle of the heteropolyblue method with some modification (Wongputtisin et al., 2012). K_2PO_4 was the standard phosphate for calibration curve preparation and estimating soluble phosphate content from the light absorbance at 820 nm.

The proximate analysis of a finished product and raw material included moisture, total ash, crude protein, crude fiber, crude fat, and carbohydrate content, which were determined using the AOAC method (AOAC, 2012) and calculated energy values. Moreover, they were freeze-dried, ground into powder, and defatted by hexane. Three grams of dry powder was mixed with 40 ml of 80% (v/v) methanol and continuously stood for two hours with intermittent shaking. The extracts were recovered by centrifugation twice at 3,500 RPM and 10,000 RPM for 15 min without temperature control. Methanol in the extract was removed by vacuum evaporation and subsequently resubstituted in DMSO at the original concentration. These extracts were subsequently analyzed for the total phenolic content (TPC) and ABTS scavenging antioxidant capacity according to the method of Suguhara et al. (2015). The content of TPC was calculated by comparing it to a standard curve of gallic acid. ABTS scavenging activity was expressed as the equivalent capacity of Trolox (positive control), and the half-maximum effective concentration (EC50) value was estimated.

Statistical analysis

The experimental results were analyzed using Minitab software (Minitab Version 21, Minitab Inc., USA). All data were expressed as the means ± standard deviations of triplicate measurements. A one-way analysis of variance (ANOVA) at the 95% significance level was used to determine significant differences ($P < 0.05$) between the means and obtain the optimized values.

RESULTS AND DISCUSSION

Effect of fermentation factors on the quality of fermented TSP

In the fermentation of TSP, many factors from nutritional, environmental and processing aspects influence the quality of the product. However, the scope of this study was to utilize TSP as the sole raw material. Thus, only three main factors, i.e., inoculum size, temperature, and fermentation time, were selected for initial investigation. In this regard, the enhancement of TSP bioavailability was one of our objectives; therefore, the resulting soluble sugar, protein, and phosphate content in the obtained products were criteria in this study. These criteria indirectly reflected the fungal growth and bioavailability of the product (solubility, digestibility, and absorbability). The results showed that soluble protein (Figure 1C) and phosphate (Figure 1C) increased along with the increase of inoculum size. At the same time, this correlation was not found in the resulting soluble reducing sugar (Figure 1A). Higher inoculation size generally shortens the lag phase of microbial growth during fermentation and also reduces the risk of microbial contamination. However, the cost of inoculum must be considered and compromised. *Rhizopus* sp. fungus can produce several enzymes responsible for polysaccharides, proteins, and phytic acid hydrolysis. Thus, a higher amount of the released protein and phosphate might correspond to the fermentation efficiency (fungal growth and nutrient bioavailability enhancement). On the other hand, the carbon source is the essential element for microbial mass production; therefore, a lowering of soluble sugar content could be observed in the treatments with higher inoculum size even though soluble protein contents of the treatments with 10^6 and 10^7 spore/g were not significantly different ($P > 0.05$) (70 – 77.32 mg/g DW). The soluble phosphate found in the treatment of 10^7 spore/g was significantly greater than in other treatments. *Rhizopus* sp. fungi are capable of phytase production (Sato et al., 2014). This enzyme hydrolyzes phytic acid (myo-inositol-1,2,3,4,5,6-hexakisphosphate), one of the antinutritional factors contained in leguminous seeds, to inositol and 6 molecules of phosphate. Thus, the higher soluble phosphate content might imply a decrease in phytic acid. According to these results, an inoculum size of 10^7 spores/g was chosen.

The effect of incubation temperature was studied at 25, 30, 35 and 40°C. The results are presented in Figure 2 (A to C). It was demonstrated that incubation at 35°C resulted in the highest content of soluble reducing sugar, available phosphate, and protein to fermented TSP. The soluble contents reached 53.82 and 272.24 mg/g DW, respectively. In 2000, Han and Nout reported that the optimal temperature for mycelium germination of *R. microsporus* on an agar medium was 40°C. However, a different growth rate of this fungus at 40°C was observed in our study. We found that the mycelium germination in the treatment of 40°C was lower than that of 35°C. It might be because of the difference in

substrate characteristics. Metabolic heat accumulation was present in the TSP substrate bed during fermentation. Thus, the actual temperature inside the TSP bed in the treatment of 35ºC might reach close to 40ºC. Furthermore, the incubation at 40ºC led to a higher evaporation rate of water, resulting in a lowering of the water activity value (a_w). The optimum a_w for *R. microsporus* is 0.995 (Han and Nout, 2000). From these results, the incubation temperature of 35°C was selected for further study.

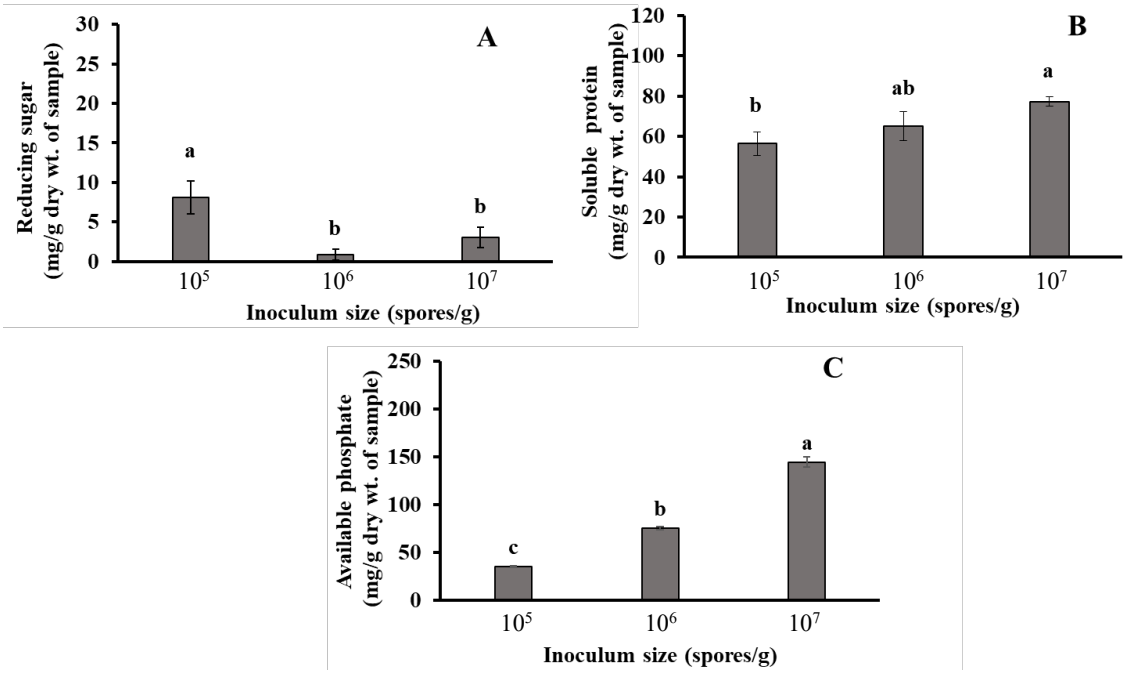

Figure 1. Effects of the inoculum size of *R. microsporus* BG5 on (A) soluble reducing sugar, (B) available phosphate, and (C) soluble protein of fermented TSP. Different letters in each experiment indicate significant differences at $P < 0.05$.

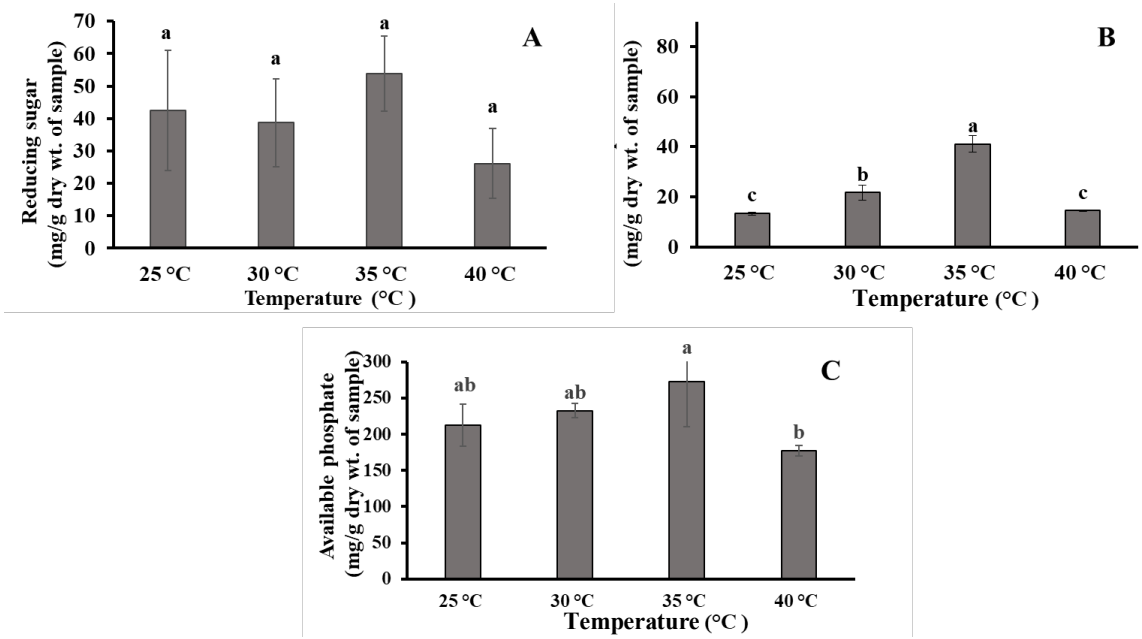

Figure 2. Effects of the incubation temperature of *R. microsporus* BG5 on (A) soluble reducing sugar, (B) available phosphate, and (C) soluble protein of fermented TSP. Different letters in each experiment indicate significant differences at $P < 0.05$.

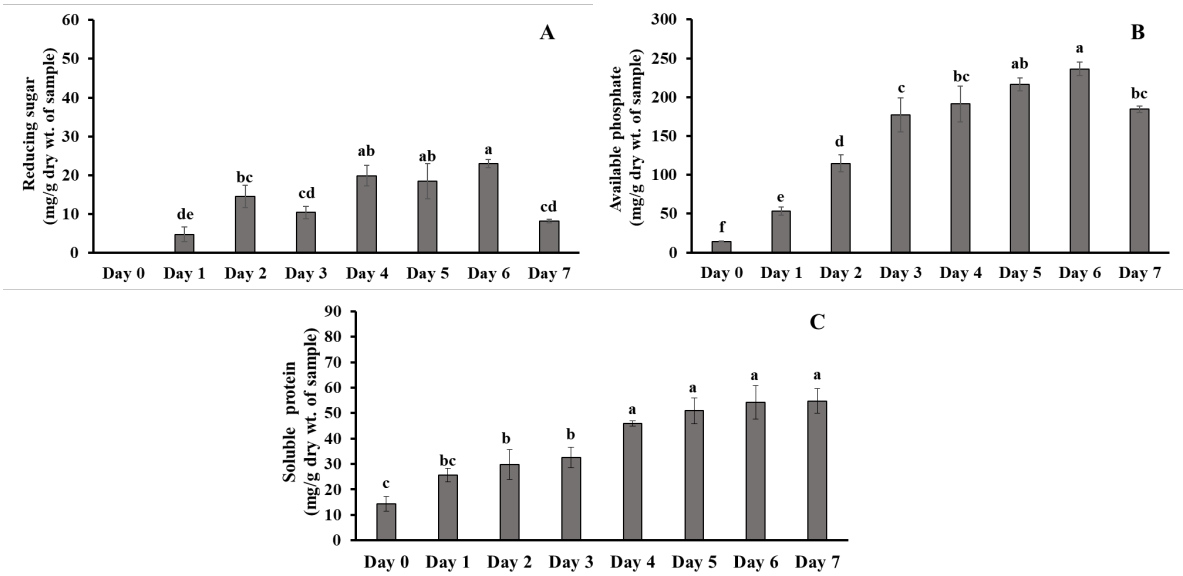

Figure 3. Effects of the fermentation time of *R. microsporus* BG5 on (A) soluble reducing sugar, (B) available phosphate, and (C) soluble protein of fermented TSP. Different letters in each experiment indicate significant differences at $P < 0.05$.

The effect of fermentation time on the soluble sugar, available phosphate, and soluble protein was investigated within the 1–7 days range; these results are shown in Figure 3. The results showed that all soluble contents increased relating to fermentation time. Soluble protein content gradually increased until day 5 and did not significantly change. The same trends were found in soluble reducing sugar and phosphate content. These contents were higher from day 0 to day 6 ($P < 0.05$) but decreased on day 7. Moreover, the capacity of ABTS radical scavenger was enhanced by approximately 10–12 folds after 5 –7 days of fermentation, referring to our preliminary test (data not shown). This finding could also indicate that TSP was a nutrient-rich substrate for microbial cultivation. However, the appearance of fermented TSP was also changed when extended fermentation time as shown in Figure 4. Color, smell, and texture were the obvious attributes that had been negatively changed and were unacceptable to consumers. The appeared caramel-like color was expected from the enzymatic browning reaction (Liu et al., 2023), while the pungent smell occurred was mainly an ammonia from protein metabolism. The softening texture could be caused by the degradation of cell structural polysaccharides and proteins. From day 0 until day 2, *R. microsporus* mycelium rapidly spread, covered, and tightly attached to the entire TSP substrate bed. This fermentation pattern was similar to that reported by Liu et al. (2023) who studied fermented dehulled soybean and found that the white mycelium of *R. oligosporus* fully covered the substrate after two days. The fermented TSP obtained in this study exhibited satisfactory attributes that met Tempe's acceptable quality standards. A good Tempe includes its compactness, with the whole surface covered by white mycelium, devoid of black spores, non-slimy, easily sliced, non-rotten, and free from ammonia odor (Nabilah et al., 2021). For the above reasons, two days of fermentation were decided to meet both nutritional and sensory acceptance. The optimal process conditions for fermented TSP production were an inoculum size of 10^7 spores/g DW of TSP, with incubation temperature at 35°C and two days of fermentation time.

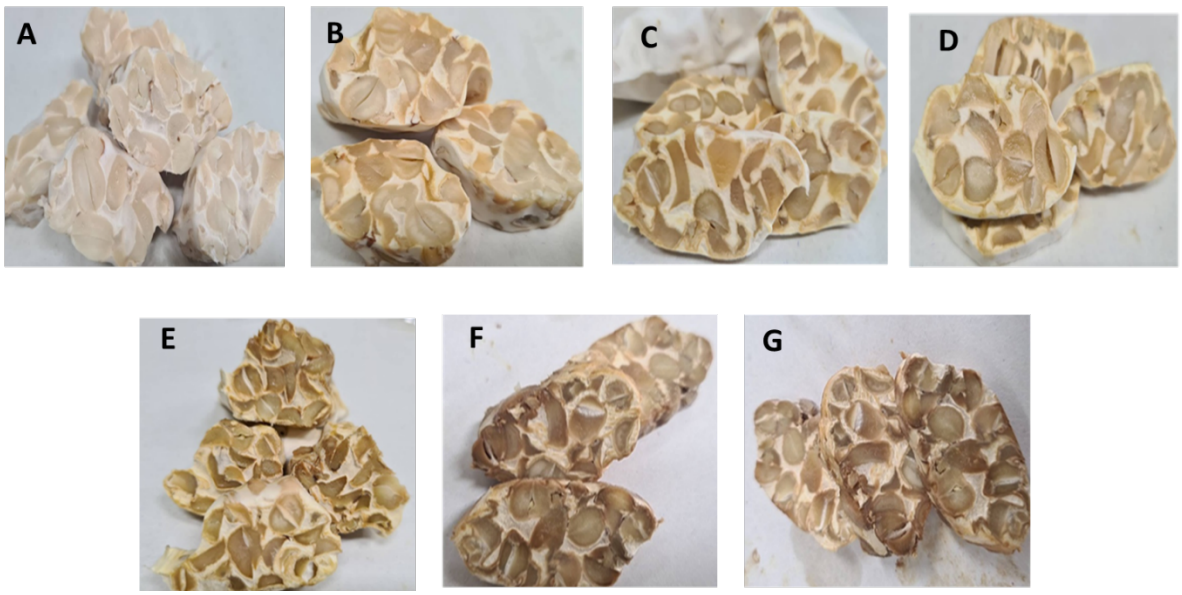

Figure 4. The appearances of fermented TSP after cultivation with 10^7 spores of *R. microsporus* /50 g, at 35◦C for 1 Day (A), 2 Days (B), 3 Days (C) 3, 4 Days (D), 5 Days (E), 6 Days (F), and 7 Days (G).

Some nutritional values of fermented TSP

The proximate analysis of fermented TSP produced under the optimal process compared to unfermented TSP was compiled in Table 1. The results demonstrated that crude protein content was 30.5% (by DW), which was enhanced by approximately 17% after fermentation. However, crude protein content increased due to the loss of carbon components during fungal metabolism, especially carbon dioxide molecules. Thus, the overall percentage of crude protein increased. According to the Indonesian National Standard (Nabilah et al., 2021), this quality met the protein standard of Tempe products, which requires a minimum protein content of 15%. The obtained protein content of our fermented TSP was also higher than the report of Matsuo (2006), who produced a peanut Tempe (Hana 17, China) by *R. oligosporus* fungus. Tempe contained 25.3% (by DW) of protein and was not different from the content of unfermented peanuts. On the other hand, fermentation slightly decreased the fat, carbohydrate, and crude fiber contents of TSP by about 3.5, 5.9 and 4.2%, respectively. At the same time, their calculated energy values were almost similar, between 538 – 539 kcal/100 g DM.

Fermented TSP contained a total phenolic content (TPC) content higher than that of TPS about 2.6 folds after fungal fermentation. These results corresponded to their ABTS scavenging antioxidant capacity. It was found that fermentation could improve the ABTS inhibition capacity (eq. mg Trolox/g DW) and the EC_{50} (mg DW/mL) of TPS approximately 1.9 folds, as the results are shown in Table 2 and Figure 5. It has long been reported and confirmed by many reports that fermentation is an effective technique for the antioxidant improvement of natural raw materials, especially cereals and leguminous seeds. The study of fermented soybean (Lo et al., 2022; Wongputtisin et al., 2007), and fermented peanut press cake (Sadh et al., 2018) are examples. Various indigenous phenolic compounds of TSP are the main compounds responsible for antioxidation activities. Radhakrishnan et al. (2014) reported the existence of many phenolic compounds, such as daidzin, genistin, quercetin, isorhamnetin, and rutin, in peanut seeds. These active compounds could be increasingly released from peanut cells because of the activity of such cell structure-degrading enzymes (cellulases, hemicellulases, and proteases). Moreover, the proteolytic activity of fungus produces short-chain peptides and free amino acids, especially arginine, tyrosine, methionine, histidine, lysine, and tryptophan that are generally accepted to be antioxidants (Wang and Mejia, 2005; Taheri et al., 2023). The reducing sugars liberated from polysaccharide hydrolysis are also accepted as potent antioxidants. The enzymatic transformation of glycoside isoflavones to aglycone isoflavones by the activity of *Rhizopus* β-glucosidase might be another mechanism to explain the increasing of antioxidant capacity. Aglycone isoflavones are the forms with higher bioactivities and bioavailability than those of glycoside forms (Baú and Ida, 2015). The antitumor, antimenopausal (female) osteoporosis and anti-aging properties, improvement of learning and memory skills of menopausal women, prevention and treatment of heart disease and diabetes are the functionalities of isoflavones (Wang et al. , 2013; Lante et al., 2018). However, this invented fermented TSP needs to investigate its specific active compounds and functionalities further before being introduced to the market with high competitiveness

Table 1. Proximate composition of tiger stripe peanut and fermented tiger stripe peanut product (%w/w)

Component	Unit	Tiger stripe peanut	Fermented tiger stripe peanut
Moisture	%	3.84	2.59
Fat	%	32.74	31.59
Protein	%	26.01	30.53
Carbohydrate	%	35.09	32.99
Ash content	%	2.29	2.27
Crude fiber	%	4.24	4.06
Energy	kcal/100 g	539	538

Table 2. The total phenolic content (TPC) and ABTS scavenging antioxidant capacity of tiger stripe peanut and its fermented product

Samples	Tiger stipe peanut	Fermented TSP
Total phenolic content (mg eq. gallic acid/g DW)	2.50 ± 0.03	6.59 ± 0.09
ABTS scavenging antioxidant capacity		
- ABTS inhibition (eq. mg Trolox/g DW)	8.29 ± 0.99	16.00 ± 1.57
- EC_{50} (mg DW/ml)	39.28	20.47

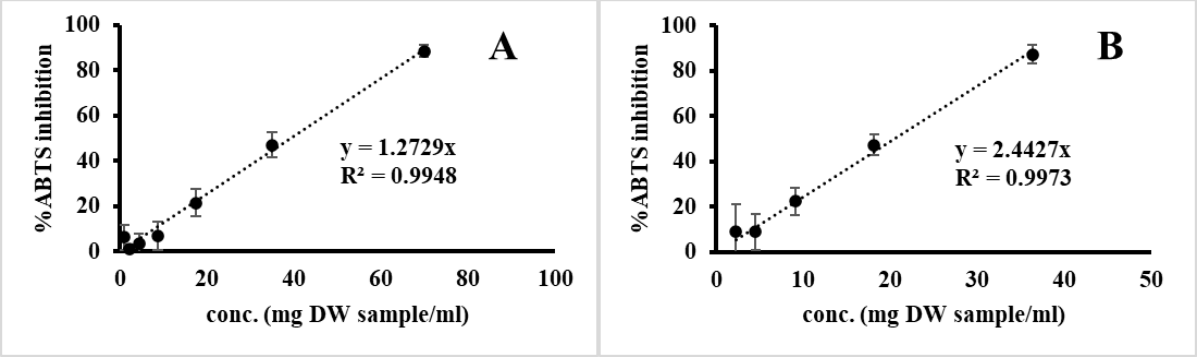

Figure 5. The ABTS inhibition capacity of tiger strip peanut extract (A) and fermented tiger peanut extract (B) at various concentrations.

CONCLUSIONS

Based on the above results, it could be concluded that the optimal fermentation conditions for fermented TSP production included an inoculation size of 10^7 spores/g, incubation at 35 ºC, and two days of fermentation period using *Rhizopus microsporus* BG5. The fermented TSP produced under these optimal conditions exhibited satisfactory nutritional values. Protein and polyphenolic contents and antioxidant capacity (ABTS scavenging activity) were significantly improved after the fermentation. This fermented TSP will be a new nutritious food product serving the healthy food market. However, further study on its active compounds, functionality, and safety are required before commercial application.

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Assessment of computer-assisted instruction multimedia: Place Around Me for enhancing English proficiency of 3rd-grade students

Wachira Lawpradit[1], Rung Mulom[1], Thanin Sinpromma[1], Prudtipong Pengsiri[1], Krittaporn Phucharasupa[2], Tantikon Phumrean[1], Thanon Waikaew[1], Phinnapha Janthasree[1] and Wanchana Joobanjong[1]*

[1]Information Technology, Faculty of Science and Agricultural Technology, Rajamangala University of Technology Lanna, Tak Campus, 41/1 Moo7, Phahonyothin Road, Muang Tak, Tak 63000, Thailand
[2]Business Information System, Faculty of Business Administration and Liberal Arts, Rajamangala University of Technology Lanna, Tak Campus, 41/1 Moo 7, Phahonyothin Road, Muang Tak, Tak 63000, Thailand

***Corresponding author**: Wanchanaj@rmutl.ac.th*

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ABSTRACT

This research aimed to evaluate teaching material in the form of computer-aided instruction multimedia named Place Around Me (PAM), which was created and developed for 3rd-grade students of English subject to assist in the class teaching activities of instructors and enhance students' English language proficiency. PAM was developed using Construct 3 and Blender software to decorate animation, scenes, and various media objects. Thirty 3rd-grade students from Anuban Tak School, Mueang Tak District, Tak province, Thailand, were assigned as sample volunteers to evaluate PAM for their satisfaction based on content aspects, appropriate media style, benefits, and use, using descriptive statistics. The effectiveness of innovations was assessed using the ratio of the efficiency of the process to the efficiency of the product. Results indicated that overall student satisfaction in content aspects based on a 5-point scale was at 4.39 or very good, media style at 4.57 or very good, media functional aspects at 4.43 or very good, and benefits and used at 4.48 or very good. The ratio of the efficiency of the process to the efficiency of the product or the effectiveness of innovations was 0.74. The results indicated that PAM is appropriate in contents, styles, functions, benefits, and practical innovation that enhances teaching and students' English proficiency.

Keywords: Computer-Assisted instruction, 3rd-grade student, Place around me

INTRODUCTION

English is a global language, and its importance is increasingly recognized in Thailand. However, the English proficiency of Thai people, particularly students, remains low. According to the National Institute of Educational Testing Service (NIETS), high school students had the lowest average English score at 43.55 in the Basic National Educational Test 2020 compared to seven other core subjects such as Mathematics, Science, etc. (NIETS, 2020). Nowadays, computer-aided instruction multimedia plays a massive role in supporting teaching and learning. Because teaching and learning styles have been changed from teacher-centered patterns, students are allowed to engage in self-learning activities and group activities that push the learners to the center of learning; the teachers would change roles and act as mentors and coordinate activities for learning, make students enjoy learning without feeling bored and can review the contents that students have learned in class as well. For these circumstances, computer-aided instruction materials have gained their roles in supporting and encouraging learners to learn at their places. At the same time, teachers can apply them to make teaching more efficient.

Yookuan (2017) reported positive outcomes from the development of computer-assisted instruction with the basic English vocabulary for the daily life of Grade 1 primary school students in Banhinwua School, Ranong province, with the effectiveness of innovations at 81.07/86.56 by meeting the set E1/E2 equal 80/80 criteria, and had statistically significantly difference ($P < 0.01$) with the higher post-study score than the pre-study score. Worawong and Charoenjitkarn (2015) mentioned that the efficiency of the computer-assisted instruction on topographies of the region lesson stand of the social study religion and culture for grade 5 primary school students of Banplongliamschool was 80.56/81.89, which met the criteria and obtained the higher post-test score than the pre-test score ($P < 0.01$). Similar results were observed by Oungthong (2018), who reported good results in using computer-assisted instruction on sharpening cutting tools for first-year students of the Department of Teacher Training in Mechanical Engineering, Faculty of

Technical Education, King Mongkut's University of Technology North Bangkok. In addition, computer-aided instruction multimedia could be adapted and modified to create new learning experiences, knowledge, and science, which are considered mediums that support the student-centered teaching and learning processes. This research addressed this issue by creating and evaluating the potential of computer-assisted instruction multimedia like Place Around Me (PAM) to enhance English learning proficiency, especially for beginning learners.

MATERIALS AND METHODS

Participations

The sample groups comprised five English teachers and 30 Grade 3 students from Anuban Tak School, Mueang Tak District, Tak, Thailand. English language teachers were required to study user requirements for teaching, teaching content, and problems encountered, and students participated in usage tests and questionnaires.

Computer-aided instruction multimedia

The computer-aided instruction multimedia PAM was developed following Vilailak (2008). Contruct 3, a game engine design, was applied to create the lesson. Then, Blender was used to create scenes, characters, and buttons for teaching materials.

Construct 3

Construct 3 is a Game Engine designed and developed by Scirra Ltd. It is accessible only at the basic creation level, but payment is required to upgrade to the full version to advance with a high-level, complex game. The full version is available at www.construct.net. The game also contains a Guided Tour button, a tutorial on how to create the game, moving objects, and animation by Goodi3 (2023).

Blender

Blender is a 3D modeling program. Like other 3D programs, it can create various 3D models, such as characters, artifacts, etc. However, Blender's advantage is that it is available for free download under the GNU GPL (General Public License). This research generated characters, models, and buildings for 3D animation using Blender: Southeast Bangkok University (2017).

System Design

The computer-aided instruction "Place Around Me" was divided into five sections (Figure 1), including the instructions section, pre-class quiz section, lesson section, after-school quiz section, and game section. The pre and post-class tests consist of 15 questions each; the lesson section has three chapters: Place, Direction, and Sentence Grammar. In the game section, there are 3 games: word-to-sentence filling games, picture-matching games, and location-based games. A storyboard is used in media story design.

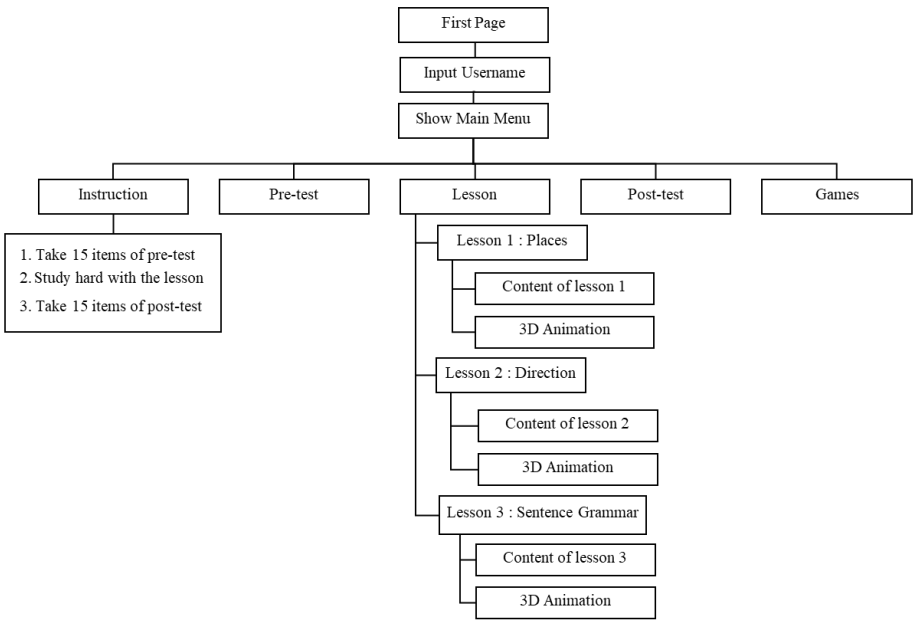

Figure 1. System design of PAM.

Storyboard

Storyboarding is a visual planning tool used in various industries, such as film, animation, advertising, and software development, to outline the sequence of events or interactions in a project. This research used a storyboard to design each scene in the lesson. The storyboard is divided into three chapters: places, direction, and sentence grammar. The storyboard examples are shown in Figure 2.

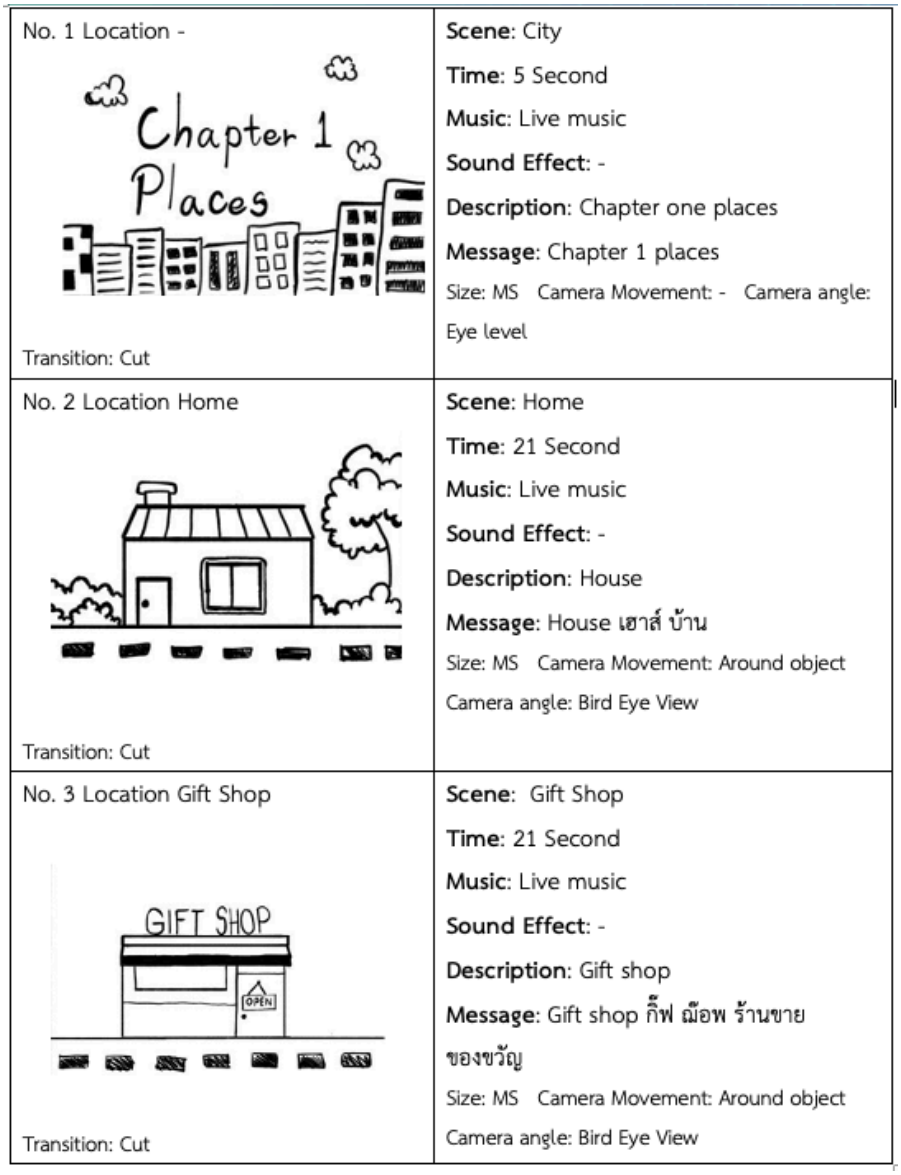

Figure 2. Storyboard example.

Data Analysis

Data were collected according to measure the satisfaction parameters of computer-aided teaching materials PAM using 5-point scales of satisfaction for assessment as follows:

A score of:

4.51 – 5.00 means that usage is at the best level or excellence
3.51 – 4.50 means that usage is very good
2.51 – 3.50 means that usage is moderate or fair
1.51 – 2.50 means that usage is low or requires improvement
1.00 – 1.50 means that usage is minimal or poor

Excellence	Good	Fair	Improvement	Poor

Then, data were analyzed using descriptive statistics, including percentage, mean, and standard deviation.

The effectiveness of innovation in teaching and learning was determined using the E1/E2 criteria; the criteria were set at 70/70, according to Chaiyong (2013). E1/E2 is a measure of the effectiveness of teaching and learning of students in the form of percentages in the E1 and E2 criteria as follows:

$$\mathrm{E1} = \frac{\bar{x}(pre - test)}{Full\ Score}\ x\ 100$$

Where: E1 is the effectiveness of innovations noted in teaching reported as a percentage of exercises or study activities during study,

$\bar{x}$ is the summary points obtained by taking the pre-test exam, and the full score variable is the full score of this pre-test.

$$\mathrm{E2} = \frac{\bar{x}(post - test)}{Full\ Score}\ x\ 100$$

Where: E2 is the effectiveness of innovations noted in teaching as a percentage of exercises and activities studied,

$\bar{x}$ is a summary point obtained by taking the post-test, and the full score variable is the full score of this post-test.

RESULTS AND DISCUSSION

Once the system is designed and computer-aided teaching multimedia is created, it can work according to the objectives. Before this system can be implemented, it must be tested using computer-aided teaching materials to know whether the various parts of the work function properly. After designing the system, teaching materials were created with the following results (Figures 3 to 10).

Figure 3. Computer-aided lessons home page.

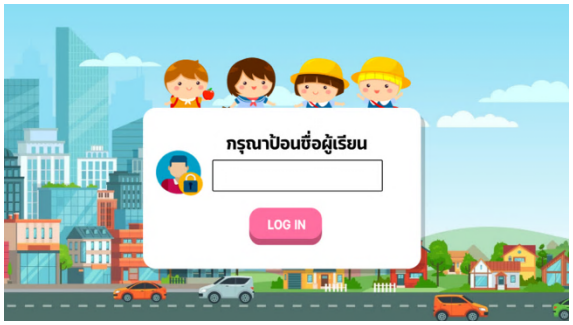

Figure 4. Enter the learner name page.

Figure 5. Menu page.

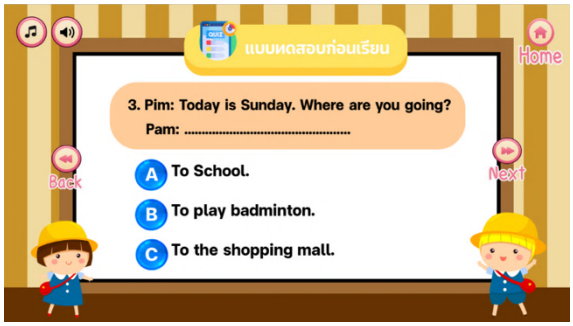

Figure 6. Quiz page.

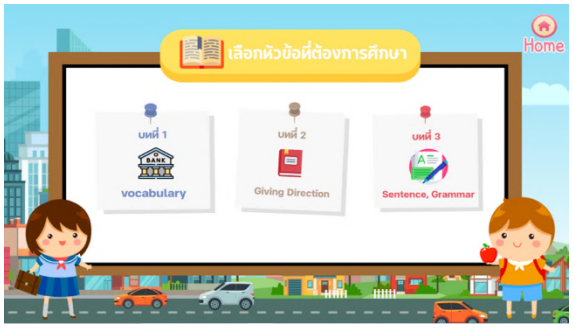

Figure 7. Topics page.

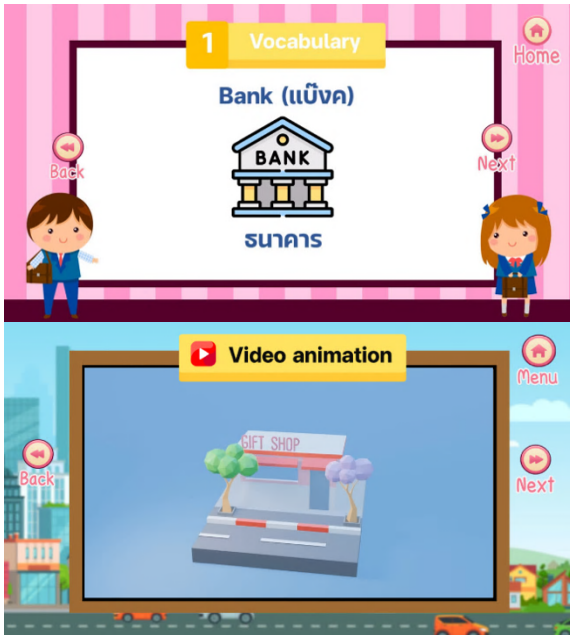

Figure 8. Lesson 1 page.

Figure 8. Lesson 2 page.

Figure 8. Lesson 3 page.

The student samples were divided into two groups by sex, including 13 males and 14 females; all were grade 3rd students. Their satisfaction results are in Table 1.

According to Table 1, the assessment of the contents was divided into five topics to observe the accuracy and appropriateness of content aspects of PAM. Topics were: 1) the content is complete, 2) the content is easy to understand, 3) the content is diverse, 4) the content meets user needs, and 5) the content is up to date. Results indicated that satisfaction with all topics was scored at very good levels, with scores of 4.37, 4.30, 4.50, 4.07, and 4.37, respectively. The overall mean was 4.39.

Table 1. Satisfaction score based on content aspects of PAM

Items	Accuracy and appropriateness level						
	Excellence	**Good**	**Fair**	**Improve**	**Poor**	**Mean**	**SD**
1) The content is complete	43.33%	50.00%	6.67%	0.00%	0.00%	4.37	0.60
2) The content is easy to understand	40.00%	50.00%	10.00%	0.00%	0.00%	4.30	0.64
3) The content is diverse	56.67%	33.33%	3.33%	6.67%	0.00%	4.50	0.84
4) Content meets user needs	56.67%	36.67%	6.67%	0.00%	0.00%	4.07	0.62
5) The content is up to date	46.67%	43.33%	10.00%	0.00%	0.00%	4.37	0.66
Overall mean						**4.39**	**0.67**

In Table 2, the assessment of satisfaction with media style was divided into five topics to observe PAM's content aspects' accuracy and appropriateness. Topics were: 1) beautiful/attractive, 2) content categorization, 3) the format and method of presenting the content, 4) font suitability and size, 5) color tone appropriateness, and 6) appropriateness of the information used in the lesson. Results indicated that all topics scored at very good levels, with 4.53, 4.53, 4.60, 4.63, and 4.47, respectively. The overall mean was 4.57.

Table 2. Satisfaction score based on the media style of PAM

Items	Accuracy and appropriateness level						
	Excellence	**Good**	**Fair**	**Improvement**	**Poor**	**Mean**	**SD**
1) Beautiful/ Attractive	60.00%	33.33%	6.67%	0.00%	0.00%	4.53	0.62
2) Content categorization.	73.33%	20.00%	6.67%	0.00%	0.00%	4.53	0.67
3) The format and method of presenting the content.	63.33%	33.33%	3.33%	0.00%	0.00%	4.60	0.50
4) Font suitability and size.	63.33%	33.33%	3.33%	0.00%	0.00%	4.60	0.50
5) Color tone appropriateness.	70.00%	23.33%	6.67%	0.00%	0.00%	4.63	0.60
6) Appropriateness of the information used in the lesson.	53.33%	40.00%	6.67%	0.00%	0.00%	4.47	0.62
Overall mean						**4.57**	**0.59**

Table 3, the assessment of satisfaction with functional aspects was divided into 4 topics to observe the accuracy and appropriateness of content aspects of PAM. Topics were: 1) computer lessons are convenient, easy to use, and not complicated; 2) ease of taking the exam in computer lessons; 3) the mini-games are easy to use; 4) 3D animations with appropriate content and easy to understand. Results indicated that all topics scored at very good levels, with 4.50, 4.43, 4.50, and 4.30, respectively. The overall mean was 4.43.

Table 3. Satisfaction score based on functional aspects of PAM.

Items	Accuracy and appropriateness level						
	Excellence	**Good**	**Fair**	**Improve**	**Poor**	**Mean**	**SD**
1) Computer lessons are convenient, easy to use, and not complicated	66.67%	23.33%	6.67%	0.00%	3.33%	4.50	0.89
2) Ease of taking the exam in computer lessons	56.67%	36.67%	3.33%	0.00%	3.33%	4.43	0.84
3) The mini-games are easy to use	63.33%	30.00%	3.33%	0.00%	3.33%	4.50	0.85
4) 3D animations with appropriate content and easy to understand	46.67%	36.67%	16.67%	0.00%	0.00%	4.30	0.74
Overall mean						**4.43**	**0.83**

In Table 4, the assessment of satisfaction with benefits and uses was divided into four topics to observe the accuracy and appropriateness of content aspects of PAM. Topics were: 1) 1) content can be put to good use; 2) media is a source of knowledge and can be referenced; 3) media that can be used in everyday life; 4) media can be used as a teaching medium as well. The results indicated that all topics scored at very good levels: 4.63, 4.30, 4.43, and 4.53, respectively. The overall mean was 4.48.

Table 4. Satisfaction score based on benefits and uses of PAM.

Items	Accuracy and appropriateness level						
	Excellence	**Good**	**Fair**	**Improvement**	**Poor**	**Mean**	**SD**
1) Content can be put to good use.	73.33%	16.67%	10.00%	0.00%	0.00%	4.63	0.66
2) Media is a source of knowledge and can be referenced.	36.67%	56.67%	6.67%	0.00%	0.00%	4.30	0.59
3) Media that can be used in everyday life.	66.67%	16.67%	13.33%	0.00%	3.33%	4.43	0.96
4) Media can be used as a teaching medium as well.	60.00%	33.33%	6.67%	0.00%	0.00%	4.53	0.67
Overall mean						**4.48**	**0.70**

To determine the effectiveness of innovation in teaching and learning using the E1/E2 criteria, according to Chaiyong (2013). Based on student pre-and post-test scores, it was found that all 30 students had an average pre-study score of 7.5 points out of 15 points and an average post-study score of 10.10 points out of 15 points. When substituting the values in the formula, it was found that the value of E1 is 50, and the value of E2 is 67.33. Then, the ratio of E1/E2 was 50/67.33, equal to 0.74, which was relatively low. In addition, the gap between E1 to E2 was 25.73%. Chaiyong (2013) mentioned that the range between E1 and E2 scores should be less than 5%; if it is more than 5%, it means

that the student activities between pre-and post-test exams are imbalanced; it indicates that the exam is more accessible or is imbalanced with the work assigned and needs to be adjusted.

CONCLUSIONS

PAM was a computer-aided multimedia instruction program developed to enhance the English proficiency of grade 3rd students at Tak Kindergarten School. After development, an assessment of PAM was conducted to evaluate satisfaction and the effectiveness of innovation. According to those results, PAM was a perfect tool and appropriate for enhancing students' English proficiency. However, student activities between pre- and post-test exams should be adjusted to increase the precision of PAM in terms of the effectiveness of innovation in teaching and learning for students due to the wide range between E1 and E2.

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Factors affecting the adoption of technology on the use of online lessons in medical care courses

Wachira Lawpradit[1], Suchart Kreuangchai[2]*, Pikul Audthiya[2], Phoomin Doungsuriya[2], Piripat Techakanta[2] and Wanchana Joobanjong[1]

[1]Faculty of Science and Agricultural Technology, Rajamangala University of Technology Lanna, Tak campus, Tak 63000, Thailand
[2]Faculty of Nursing, Boromarajonani College of Nursing, Nakhon Lampang, Lampang 52000, Thailand

***Corresponding author**: suc2513@gmail.com

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ABSTRACT

This research aimed to examine the factors affecting the acceptance of technology in the use of the online learning media system at Lampang Boromrajonani College of Nursing, Lampang, Thailand. The researcher designed and developed an online learning media system based on the principles of software development life cycle (SDLC) and applied it to 116 fourth-year nursing students, divided into 4 males and 112 females, evaluated using a questionnaire on its utilization and technology acceptance regarding the theory of technology acceptance model (TAM). The independent variables were perceived usefulness and perceived ease of use, and the dependent variable was the intention to use. Data on satisfaction were analyzed using descriptive statistics, while data on technology adoption were analyzed using multiple regression analysis. Results indicated that the parameter on suitability of the media had an average of 4.56 $\pm$ 0.56, indicating the highest satisfaction based on a 5-point scale. Moreover, the parameter on media efficiency had an average of 4.50 $\pm$ 0.61, indicating high satisfaction based on a 5-point scale. For technology adoption, it could be summarized as the equation: $Yt = 0.697 + 0.573X_1 + 0.277X_2$ where Yt = technology adoption, X_1 = perceived usefulness, and X_2 = perceived ease of use with P-value = 0.000, and $R^2 = 0.567$, indicating fair technology adoption among users.

Keywords: technology acceptance model, TAM, online lesson, medical care course

INTRODUCTION

Boromrajonani College of Nursing, under the Royal Institution Krathong Public Health, plays crucial role in producing competent nurses who adhere to the standard framework for nursing degrees and serve the Thai community in northern Thailand. In this regard, the college has designed and provided both theoretical and practical processes in teaching and learning to simultaneously develop learners' cognitive, psychomotor, and affective learning to equip them with appropriate 21st-century skills. These include 1) morality and ethics, 2) knowledge, 3) intellectual skills, 4) interpersonal skills and responsibility, 5) numerical analysis skills, and 6) professional practical skills. In past pedagogy, nursing students' learning before practicing at the hospital required them to use the search method for self-study to gain additional knowledge from the library. However, the information provided was only in terms of texts and images, which made it impossible to acquire as much knowledge as it should, especially in basic medical care courses for nursing students.

Media integration in the learning process is gaining recognition for its role in enhancing educational experiences and outcomes. It not only boosts engagement, understanding, and accessibility but also caters to diverse learning styles and promotes digital literacy. This approach creates a dynamic and interactive learning environment, equipping students to tackle the challenges of the modern world and developing students' knowledge and skills to be suitable for learning in the 21st century while students can easily access and learn effectively from electronic online learning materials (Gerdruang et al., 2021). However, effective electronic online learning materials in medical care courses are still limited. Developing learning materials through multimedia technology for nursing students of the Boromrajonani College of Nursing in basic medical care courses is a challenge to support those students with easy access and the ability to learn at their places through the internet network.

This research aimed to develop learning materials through multimedia technology via an online website for introductory medical care courses and examine the factors affecting their acceptance

and use at Lampang Boromrajonani College of Nursing, Lampang, Thailand.

MATERIALS AND METHODS

Research Process

The research developed learning materials through multimedia technology and an online website in the basic medical care course according to the Information System Development Life Cycle (SDLC) in the brief Radack (2009) and conducted a study on factors affecting the adoption of technology in the use of online lessons in medical care courses. The SDLC method is shown in Figure 1, and the research process in Figure 2.

From Figure 1, SDLC is separated into 5 phases: initiation, development and acquisition, implementation, operations and maintenance, and disposal. This research was about developing the system according to this SDLC by the following steps: First, researchers collected user requirements and important data from users, students, and lecturers. Second, the system was analyzed and designed physically and logically. Third, the system was created using the PHP, HTML, JavaScript, and Bootstrap frameworks. Fourth, the system checked the errors for corrections. Finally, the new system was used in the medical care classroom. After using the system, researchers collected the satisfaction data of the online lesson system from 116 fourth-year nursing students. Then, the Technology Acceptance Model (TAM) of Davis (1989), as explained by Luenam (2011) was applied by following the concept framework. The research hypothesizes that there were two factors (Figure 2) affecting adoption of technology utilization as follows:

Factor 1: Perceived usefulness: This might affect the user's acceptance of technology and intention to use the online lesson system for primary medical care courses.

Factor 2: Perceived ease of use: This might affect and relate to users' acceptance of technology.

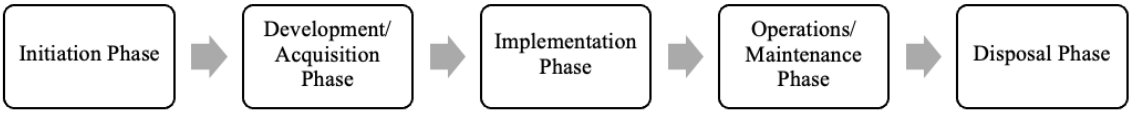

Figure 1. Software development life cycle.

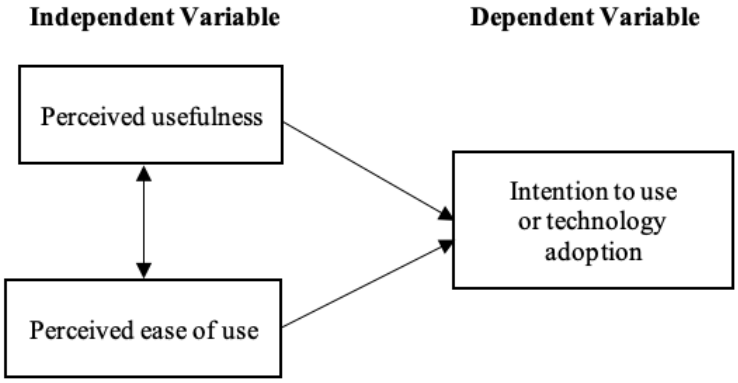

Figure 2. Factors affecting Intention to use or technology adoption.

Participants

116 fourth-year nursing students at Boromrajonani College of Nursing, including 4 males and 112 females, participated as volunteers to use the developed multimedia technology to learn materials in the basic medical care courses and answer the questionnaire.

Contents used to develop online lessons

This research has contents related to primary medical care, divided into ten topics as follows:

Topic 1: Preparing to clean the wound (scrub wound)
Topic 2: Selection of materials and equipment for stitches
Topic 3: Incision and drainage
Topic 4: Single interrupted suture
Topic 5: Vertical mattress suture
Topic 6: Half-buried horizontal mattress suture
Topic 7: Nail avulsion
Topic 8: Emergency intravenous fluid administration
Topic 9: Preparing equipment for doctors for emergency intubation
Topic 10: Basic cardiopulmonary resuscitation according to AHA 2020 guidelines.

Research Statistics

We evaluated the system after its use over a one-semester period with 116 nursing students. The degree of agreement or acceptance was used on a 5-point scale according to Likert's scale related to Jebb et al. (2021) as follows:

A score of 4.51 – 5.00 means the highest level of agreement or acceptance
3.51 – 4.50 means a high level of agreement or acceptance
2.51 – 3.50 means a moderate level of agreement or acceptance
1.51 – 2.50 means disagreement or unacceptance
1.01 – 1.50 means very disagreement or poor unacceptance

Data were analyzed using descriptive statistics, including mean and standard deviation for satisfaction with using the system, while technology acceptance was analyzed using multiple regression using SPSS statistical software version 26 (IBM Corp., New York, USA) with a significance level of $P<0.05$.

RESULTS AND DISCUSSION

The multimedia online lessons in medical care courses

This research develops the online lesson system following the Software Development Life Cycle (SDLC) and finally finishes the development of the system with the following figures:

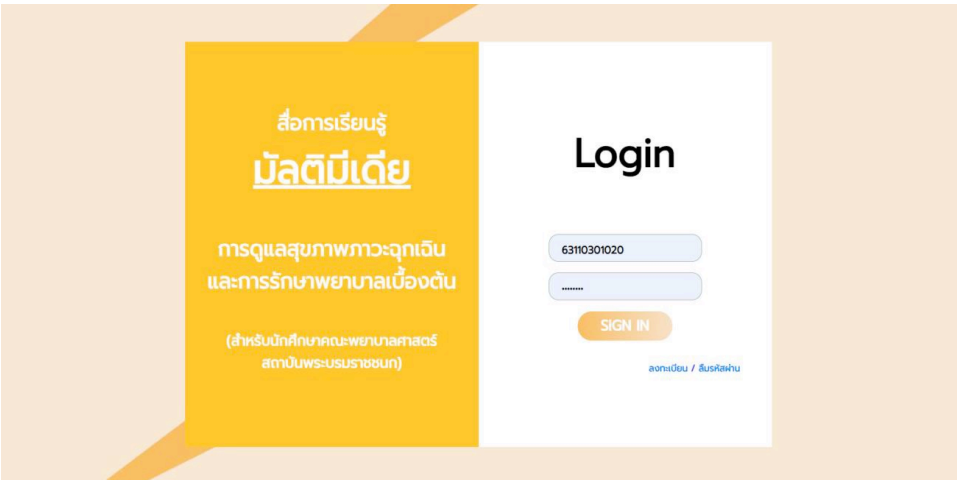

Figure 3. Login page.

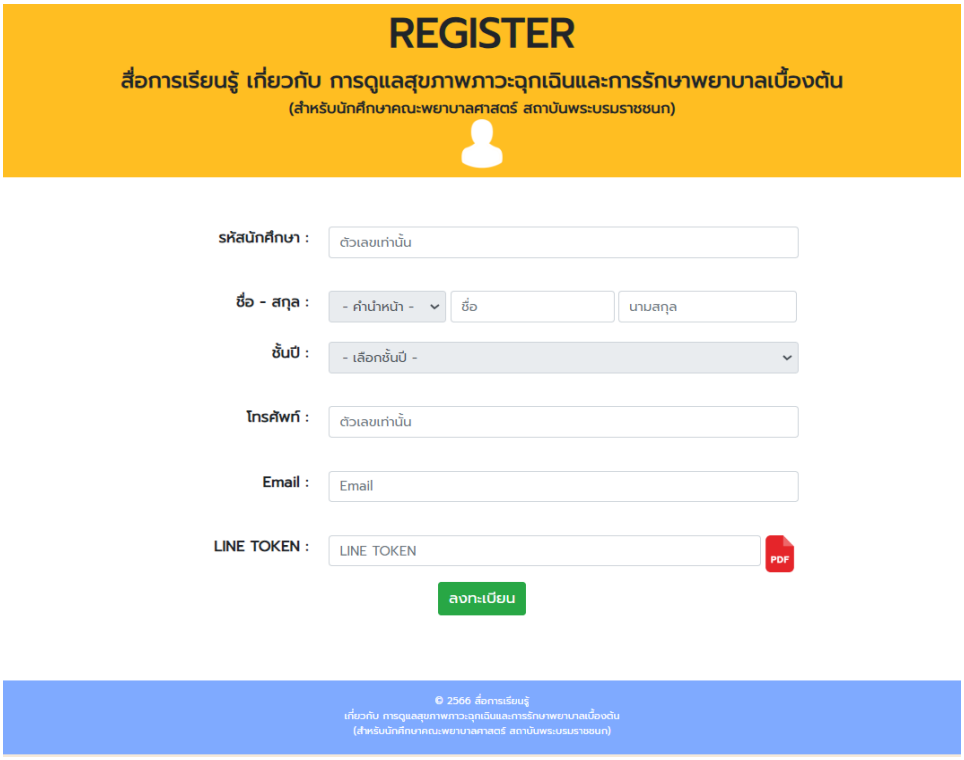

Figure 4. Register page.

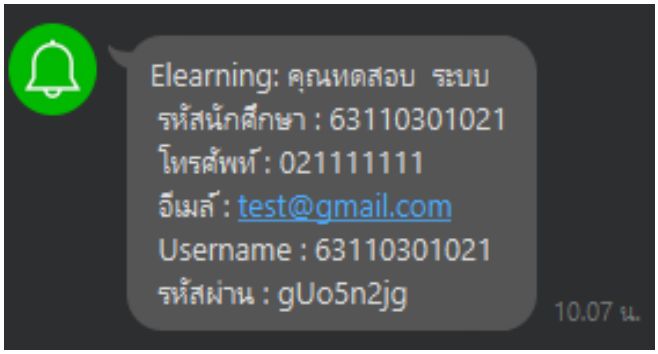

Figure 5. LINE notification.

Figure 6. Course page.

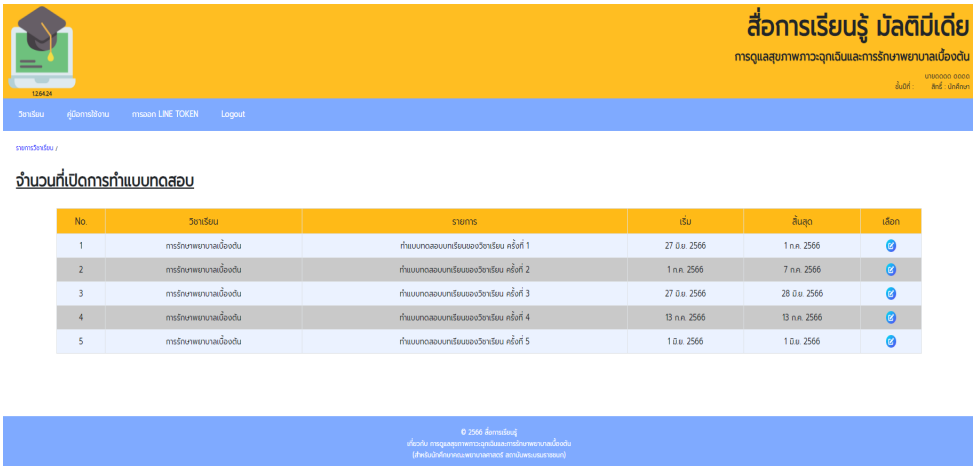

Figure 7. Examination page.

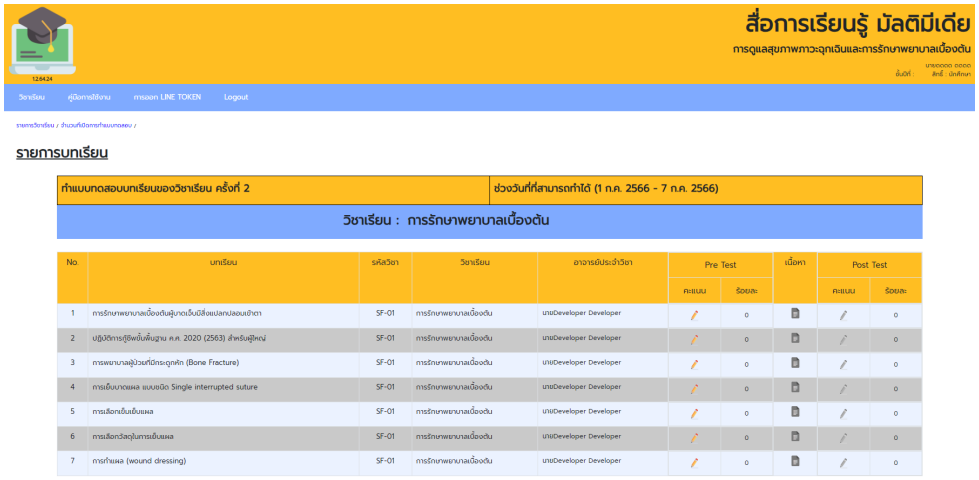

Figure 8. Course summary page.

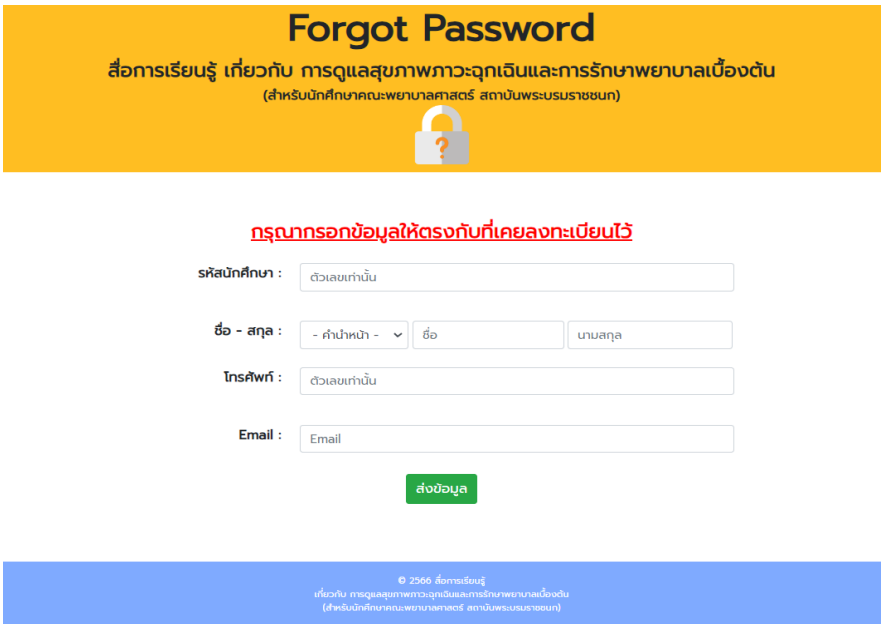

Figure 9. Forgot password page.

The analysis of factors affecting the adoption of technology in the use of online lessons in medical care courses was divided into two topics. First, the analysis of satisfaction with using the system was designed to examine the assessment of the system after use. Second, the analysis of technology acceptance towards the use of online lessons in medical care courses based on the technology acceptance model (TAM) was designed to predict the user's need to use the system again.

Satisfactions of using the system

After the development of the online lesson system, the medical course was completed. The system has been deployed for nursing students. The assessment of the system is divided into two parts: the appropriateness of the online lesson system and the efficiency of the online lesson system. According to the method, the results of the appropriateness of the online lesson system are shown in Table 1, and the efficiency of the online lesson system is shown in Table 2.

Table 1. Factor: perceived usefulness factor of the online lesson system

Items	Mean	SD	Outcome
1. The content in multimedia about the nature of the subject is easy to read and clear	4.50	0.61	Highest
2. The content sequencing is easy to understand	4.50	0.56	High
3. The content in multimedia media is substantive, accurate, and meets the learning objectives that are very suitable for students	4.56	0.60	Highest
4. The amount of content in each topic is short, concise, and clear	4.47	0.58	High
5. Students understand the material easily and can learn better	4.64	0.55	Highest
6. Content in multimedia knowledge is transferred by explaining examples clearly and easily	4.52	0.58	Highest
7. Encourage students to think critically, make decisions, solve problems, and develop more skills	4.60	0.56	Highest
8. Learners can access self-paced learning through multimedia anytime, anywhere	4.62	0.50	Highest
9. Learning multimedia can be used for teaching, learning, and practical application in daily life.	4.63	0.55	Highest
10. Before and after learning quizzes are consistent with the content	4.61	0.54	Highest
11. Test processing is quick and convenient, making students aware of their abilities and what they need to develop	4.56	0.56	Highest
Overall mean	**4.56**	**0.56**	Highest

Highest = highest level of agreement or acceptance. High = high level of agreement or acceptance.

According to Table 1, we found that online lessons in medical treatment courses were overwhelming. In terms of the suitability of the lesson system, the overall average score level was 4.56, and the average standard deviation was 0.56 (Mean = 4.56, SD = 0.56), which was at the highest agreement or acceptance level. When considering all topics individually, 11 topics were found appropriate. The assessment results are in the highest agreement. The most-rated scale topic was that students understood the material easily and could learn better (Mean = 4.64, SD = 0.55). The minimal rated topic was the amount of content in each topic that is short, concise, and clear (Mean = 4.47, SD = 0.58). Perceived usefulness refers to a person's level of belief that the introduced technology can help increase work efficiency (Sabbunyat, 2019)

Table 2 shows that online lessons on medical treatment courses are overwhelming. In terms of the effectiveness of the lesson system, the overall average score level is 4.50, and the average standard deviation is 0.61 (Mean = 4.50, SD = 0.61), which is at the level of very agreeable. When considering all topics individually, 11 topics were found appropriate. The assessment results are between very and maximum. The most-rated scale topic is multimedia media, which has a connection with content, essence, and a step-by-step summary (Mean = 4.62, SD = 0.52). The design, layout, use of multimedia media, and screen are proportional and beautiful (Mean = 4.40, SD = 0.64). Perceived ease of use refers to a person's level of belief that using technology will not require much effort. If the technology is simple, there will be no obstacles. If the system is complex, it can create a positive attitude towards service users (Himarat, 2016).

Table 2. Factor: perceived ease of use of the efficiency of the online lesson system

Items	Mean	SD	Outcome
1. The design, layout, use of multimedia media, and screen are proportional and beautiful	4.40	0.64	High
2. The establishment of various menus of multimedia media is clear and interesting	4.42	0.62	High
3. The colors of multimedia are appropriate	4.44	0.66	High
4. The font size in multimedia media is easy to read and clear	4.43	0.63	High
5. Multimedia media is beautiful	4.48	0.64	High
6. Multimedia media uses creative thinking	4.53	0.61	Highest
7. The presentation style is beautiful and interesting	4.42	0.66	High
8. The amount of material in each subject matter is accurate, clear, and up-to-date	4.55	0.61	Highest
9. The arrangement of each topic is easy to read and clear	4.60	0.59	Highest
10. Multimedia media has a connection with content, essence, and a step-by-step summary	4.62	0.52	Highest
11. Multimedia media showing images, videos, and audio commentary are clear	4.59	0.56	Highest
Overall mean	**4.50**	**0.61**	High

Acceptance towards the use of online lessons in medical care courses based on TAM

In the process of analyzing the adoption of technology towards online lessons on medical care courses according to the technology acceptance model (TAM), the research uses a multiple regressive analysis of technology adoption to intent to use online lessons, defining the following points:

Perceived usefulness

1. In the process The content in multimedia media is substantive, accurate, and meets the learning objectives that are very suitable for students.
2. Content in multimedia Knowledge is transferred by explaining examples clearly and easily.
3. Encourage students to think critically. They can learn more skills.
4. Learning multimedia can be used for teaching, learning, and practical application in daily life.
5. Before and after quizzes from multimedia are consistent with the content.

Perceive ease of use

1. The content in multimedia about the nature of the subject is easy to read and clear.
2. Content sequencing is easy to understand.
3. The amount of content in each topic is short, concise, and clear.
4. Students understand the material easily and can learn better.

For technology adoption, it could be statistical inference using the multiple regression equation to predict the value of the change in the intention to use the online lesson system, which is equal to a percentage of 56.70. This analysis found the elements of technology adoption (Y_t) responded to perceived usefulness. (X_1) and perceived ease of use. (X_2), which were statistically significant with detail in Table 3 and could be illustrated in a multiple linear regression equation as follows:

$$Y_t = 0.697 + 0.573X_1 + 0.277X_2 \quad \text{with } R^2 = 0.567$$

Table 3. Multiple regression equation of technology adoption vs. perceived usefulness and perceived ease of use for online lesson systems

Items	B	SE	Beta	t	P-value
Constant	0.697	0.319		2.183	0.031
Perceived usefulness (X_1)	0.573	0.102	0.530	5.642	0.000
Perceived ease of use (X_2)	0.277	0.097	0.269	2.867	0.005
$R^2 = 0.567$, SEE = 0.320, F = 74.003, P-value = 0.000					

The equation suggests that the adoption of technology is relatively related to perceived usefulness and perceived ease of use ($P < 0.05$). However, the relationship is quite weak due to a moderate low in R^2. In terms of technology adoption, the results were in agreement with Yeemali (2017), who reported a relationship between perceived usefulness and perceived ease of use in terms of adoption or intention to use a technology (movie application and series) in Bangkok.

CONCLUSIONS

An online learning media system based on the principles of the software development life cycle was developed for introductory medical care courses and benefits students at Lampang Boromrajonani College of Nursing, Lampang, Thailand. Factors that affect the adoption of technology are perceived usefulness and perceived ease of use, which have shown a linear relationship. Related multimedia technology may require further development to enhance learning styles and promote digital literacy that is appropriate for 21st-century skills.

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Effect of avocado pulp puree on the quality of Thai-style custard cake

Donporn Wongwaiwech[1*], Monthana Weerawatanakorn[2], Boondarick Rodbumrung[3], Surasawadee Paliphot[3], Supalak Wongkaunkaew[1], Areeya Aroon[1] and Surasit Tongsa-ard[1]

[1]Department of Agro-Industry, Faculty of Science and Agricultural Technology, Rajamangala University of Technology Lanna, Tak Campus, 41/1 Moo 7 Paholayothin Road, Mai Ngam, Muang, Tak 63000, Thailand
[2]Department of Agro-Industry, Faculty of Agriculture, Natural Resources and Environment, Naresuan University, 99 Moo 9, Tha Pho, Phitsanulok 65000, Thailand
[3]Department of Science, Faculty of Science and Agricultural Technology, Rajamangala University of Technology Lanna, Tak campus, 41/1 Moo 7 Paholayothin Road, Mai Ngam, Muang, Tak 63000, Thailand

***Corresponding author**: donporn@rmutl.ac.th*

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ABSTRACT

The study aimed to evaluate the effects of replacing coconut milk with avocado pulp puree in a healthy Thai-style custard cake (Khanom Mor Kaeng) on their physicochemical, microbiological, and sensory properties. The results showed that Thai-style custard cake at different substitution levels of avocados: coconut milk (0, 25, 35, 45, and 55%) appeared darker and more green and yellow. Thai-style custard cake at a 35% substitution level of avocado pulp puree was selected with the highest scores in color, aroma, texture, taste, and overall acceptability at 7.23, 7.33, 7.36, 7.30, and 7.36, respectively. The calorie content, total carbohydrate, total fat, and saturated fatty acid were lower than those of the original product by 3.21, 0.82, 10.01, and 25.29%, respectively. However, the unsaturated fatty acid and crude fiber of the developed product were increased by 1.6-fold and 12.23%, respectively, when compared to the original product.

Keywords: Thai-style custard cake, avocado, coconut milk, avocado pulp puree, sweetener

INTRODUCTION

Obesity represents a global public health problem that affects almost every country in the world, including Thailand in this present time. Obesity is a non-communicable disease (NCD), and it is the leading cause of other serious diseases such as diabetes, stroke, heart disease, cardiovascular diseases, and metabolic syndrome. (Budreviciute et al., 2020; Jaoua et al., 2020) The World Health Organization (WHO) reported that the heart disease group and vascular disease group have been the number one cause of death globally since 2016 (Department of Disease Control, 2022). In 2022, the number of Thai obese patients was reported to be 9 percent of the total Thai population, or 6 million people, which was the second highest number after Malaysia, and the number has continued to increase. According to Puntiya (2020) stated that there were 17.9 million people died from obesity, accounting for 31% of the total death rate. The cause of obesity death rate could relate to regularly consuming high-fat and sugar in Thai food and desserts. Thai desserts are mainly comprised of coconut milk and sugar, which provide high energy. One of the famous Thai desserts that people usually have is Thai custard with mung beans or Khanom Mor Kaeng Thua.

Khanom Mor Kaeng Thua, or its original name, Khanom Mor Thong, was first made by Thao Thong Kip Ma (Maria Guyomar de Pinha) in the reign of King Narai, the Great. When she started promoting the dessert recipe to other people, one of them was a group of local people from Phetchaburi province who were her kitchen assistants. Therefore, the most famous and authentic recipe is in Phetchaburi. Nowadays, the recipe for making Khanom Mor Kaeng Thua has been adapted from palm sugar to jaggery palm sugar, increasing the sweetness and unique smell, which will help the dessert have a smooth texture, brown surface, soft texture, and sweet taste. The main ingredients in 100 grams of Khanom Mor Kaeng Thua is associated with a high content of sugars, flour, and coconut milk, which provide 223 calories of energy, 6.20 grams of protein, 26.60 grams of carbohydrates, 21.00 grams of sugar, and 10.10 grams of fat which is saturated fatty acid up to 4,517 milligrams (Jongjaithet et al., 2009). As a sequence of desserts provides high fat, many researchers have experimented with improving the recipe to be more nutritious and healthier by reducing the amount of sugar, replacing sweeteners of Maltitol, Sucralose, and fatty acids with other substances such as replacing coconut milk with Grains milk and avocado. Sukonthara (2016)

conducted a study on developing Khanom Mor Kaeng germinated Riceberry to reduce sugar. Chompoorat (2022) conducted a study on developing low calories in Khanom Mor Kaeng with no cholesterol from coconut milk oil and eggs. Sanon et al. (2009) studied reduced calories in Khanom Mor Kaeng and improved fatty acid proportion with sucralose and cream from grain milk.

Avocado is a fruit that provides an excellent essential source of many nutrients and minerals for the body, such as Potassium, Phosphorus, Calcium, Manganese, Vitamin A, Vitamin C, and Vitamin D (Kadam and Salunkhe, 1995). Avocado pulp has a high-fat content of up to 2735% (Phungbunhan et al., 2021), which is a monounsaturated fatty acid (MUFA) 10% (Okobi et al., 2013). This type of fatty acid helps reduce low-density lipoprotein (LDL) levels (Wang et al., 2015), increasing the blood's high-density lipoprotein (HDL) levels and decreasing the risk of having heart disease (Okobi et al., 2013). Tak province is another important avocado cultivation area in Thailand. Most of the plantation area is more than 1,369 rai or 219 hectares, and the total production is about 800–1,200 tons annually. These large amounts of harvesting could lead to market oversupply, low prices, and waste. For these reasons, the researchers aimed to develop the Khanom Mor Kaeng Thua recipe by replacing fatty acids in coconut milk with avocado pulp and utilizing maltitol and sucrose instead of sugar to enhance nutrition, reduce the energy to the body, and be the alternative for good healthy.

MATERIALS AND METHODS

Materials

Thai avocados (PP08 varieties), and other food-grade ingredients (eggs, granulated sugar, palm sugar, coconut milk, and salt) were purchased from the local market (Tak, Thailand). The avocado fruits were transported to the laboratories in Rajamangala University of Technology Lanna Tak and allowed to ripen for 5 days in a room at ambient temperatures before use. The avocados were washed and separated pulp from seed and peel. The avocado pulp was mashed into a paste and kept in polyethylene bags at –20°C until use.

Thai-style custard cake preparation

Thai-style custard cake was prepared following the formulations as shown in Table 1 with the addition of five different concentrations of PP08 avocado pulp puree (0, 25, 35, 45, or 55%). The ingredients were mixed in the smoothie blender (OTTO, BE-127A, Thailand); first, eggs, granulated sugar, palm sugar, and salt were mixed at low speed for 2 minutes; steamed peeled-split mung bean, avocado pulp puree, and coconut milk were added to the mixture. It was then mixed until it was uniform in consistency. The batter was then placed in baking tins and baked in the baking oven (Electrolux, EOT70DB, Thailand) set at 200°C for 40 minutes. The Thai-style custard cakes were then cooled, wrapped in plastic bags, and stored at room temperature for further analysis.

Table 1. The Thai-style custard cake formulation with five different proportions of coconut milk and avocado pulp puree

Ingredients	Formulations (%)				
	F1 (0%)	F2 (25%)	F3 (35%)	F4 (45%)	F5 (55%)
Egg	18	18	18	18	18
Granulated sugar	12	12	12	12	12
Palm sugar	7	7	7	7	7
Salt	1	1	1	1	1
Steamed peeled-split mung bean	26	26	26	26	26
Coconut milk	36	27	23.40	19.80	16.20
Avocado pulp puree	0	9	12.60	16.20	19.80

Color

A chromaticity instrument (Hunter, Miniscan EZ 4500L Spectrophotometer, USA) was employed to measure the surface and inner color of the samples. A white and black standard board was used for calibration. The color values were expressed as L* (whiteness/darkness), a* (redness/greenness), and b* (yellowness/blueness). Three samples were analyzed for each substitution level, and each sample was analyzed at three different locations.

Texture analysis

A TA-XT pules Texture Analyzer (Texture Analyzer, TA-XT pules, Stable Micro Systems Ltd., England) was used to measure the hardness, adhesiveness, springiness, cohesiveness, and chewiness of the samples. The samples were cut into

a cube (5.0 cm*5.0 cm*1.5 cm). A 5 mm cylindrical probe was used to cut the samples. The crosshead speed was set at 1 mm s-1, and the probe traveled 75% of the depth into the sample in the first stage (Murdia, 2010).

Calorific and nutritional value

The determination of calorific and nutritional value was performed by indirect calorimetry: Using the food composition table developed by the nutritive values of Thai foods and FoodData Central (Bureau of Nutrition, 2018; United Department of Agriculture, 2020), the sample was calculated for energy (kcal), protein (g), lipid (g), carbohydrate by difference (g), dietary fiber (g), and saturated and unsaturated fatty acids in mg.

Sensory evaluation

The sensory characteristics of Thai-style custard cake were evaluated by 100 untrained panelists aged between 25 and 70. The samples were prepared the day before testing. The samples were cut into 2.0 cm*2.0 cm*1.5 cm cubes. In total, five custard cake samples were presented simultaneously on a serving plate. Each sample was coded with a 3-digit random number. The nine-point hedonic scale used to evaluate color, aroma, taste, and overall acceptability had a verbal interpretation where 9 denoted extremely, 5 denoted neither like nor dislike, and 1 denoted dislike extremely. For the texture parameter, 9 denotes extremely smooth, 5 denotes neither smooth nor rough, and 1 denotes extremely rough.

Statistical analysis

All experiments were conducted in triplicate. Differences between means were determined by one-way analysis of variance (ANOVA) and Duncan's post-hoc test (using SPSS statistical software version 26, IBM Corp., New York, USA) with a significance level of $P < 0.05$.

RESULTS AND DISCUSSION

Effect of substitution level on color

Figure 1 and Table 2 show the physical appearance and the results of color tests, respectively. For the five custard cakes, L* and a* values decrease significantly with increased avocado pulp puree (APP) content. By contrast, the b* value of the surface and inner of custard cakes increased significantly. There was a significant difference in corresponding color values among the custard cakes containing different APP substitution levels. This indicates that samples with more APP are darker, greener, and more yellow. The decrease in the L* value is attributed to the browning reaction. Cut avocados turn brown due to an enzyme present in the avocado called polyphenol oxidase (PPO) that oxidizes polyphenols, causing a brown color to develop. PPO catalyzes the oxidation of phenols to quinones that subsequently polymerize into brown pigments (Zhou et al., 2016). However, these phenomena can be avoided using anti-browning agents such as citric acid, ascorbic acid, and oxalic acid (Suttirak and Manurakchinakorn, 2010). The changes in a* and b* values were caused by plant pigments in avocado pulp, including chlorophylls and carotenoids, especially lutein (Ashton et al., 2006). A similar result has been reported for muffins incorporated with avocado pulp puree (Narul Ain et al., 2016).

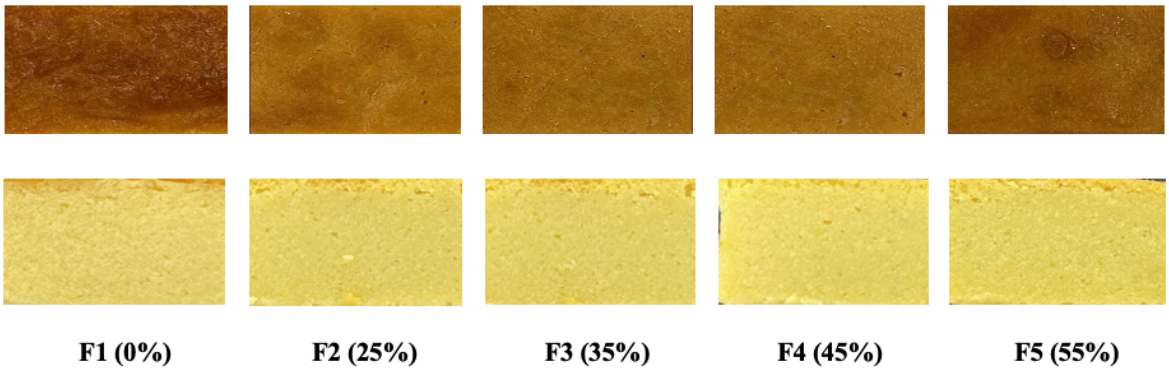

Figure 1. Effect of avocado pulp puree substitution on the physical appearance of Thai-style custard cake.

Table 2. Effect of avocado pulp puree substitution on color value of Thai-style custard cake

Color value	Sample				
	F1	F2	F3	F4	F5
Surface of Thai-style custard cake					
L*	50.08 ± 0.58^{a}	48:89 ± 0.58^{a}	43.68 ± 0.58^{b}	42.45 ± 0.58^{b}	37.18 ± 0.58^{c}
a*	21.77 ± 0.57^{a}	16.38 ± 0.57^{b}	15.84 ± 0.57^{b}	13.51 ± 0.57^{c}	12.07 ± 0.57^{c}
b*	31.36 ± 0.12^{d}	33.82 ± 0.12^{c}	35.26 ± 0.12^{b}	35.44 ± 0.12^{b}	40.60 ± 0.12^{a}
Inner of Thai-style custard cake					
L*	63.51 ± 0.84^{a}	56.21 ± 0.84^{b}	53.59 ± 0.84^{c}	52.53 ± 0.84^{c}	52.92 ± 0.84^{c}
a*	7.71 ± 0.34^{a}	6.83 ± 0.34^{b}	5.68 ± 0.34^{c}	5.64 ± 0.34^{c}	5.24 ± 0.34^{d}
b*	26.18 ± 0.05^{c}	28.67 ± 0.05^{b}	28.54 ± 0.05^{b}	31.72 ± 0.05^{a}	31.75 ± 0.05^{a}

Mean ± standard deviation (n=3). Values with different superscript letters in a row differ significantly ($P < 0.05$). F1 = control, F2 = 25% APP, F3 = 35% APP, F4 = 45% APP, F5 = 55% APP. APP = avocado pulp puree.

Effect of substitution level on textural properties

The texture analysis results including hardness, adhesiveness, springiness, cohesiveness, and chewiness of the Thai-style custard cakes (TSC) are shown in Table 3. The hardness and chewiness ranged from 123.44–131.17 g and 56.43–65.62 g, respectively, and the use of APP made the TSC significantly harder and chewier. Usually, fat substitution, either partially or fully, produces harder-baked products (Nurul et al., 2016). Many studies have reported this on the incorporation of fat replacers such as sesame oil, peanut butter, and extra virgin olive oil as margarine substitutes in baked goods (Matsakidou et al., 2010; Sadaf et al., 2013; Sowmya et al., 2009). The chewiness of the TSC increased with the addition of APP and reached a maximum of 55% fat level. The avocado contains about 6.7 g/100 g of fiber (U.S. Food and Drug Administration, 2018), and this factor could also contribute to a slightly chewier texture in the fat-replaced samples. On the other hand, increasing APP substitution level significantly decreases the adhesiveness, springiness, and cohesiveness, ranging from –59.38 to –46.47 (g/s), 0.90–0.96 mm, and 0.47–0.51, respectively. A similar result was reported by Marina et al. (2016). The results showed that the butter cakes became harder while springiness and cohesiveness decreased with increasing APP substitution levels. Rodríguez-García et al. (2014) also observed a decrease in springiness when the cake was replaced with inulin as a fat replacer.

Table 3. Textures of Thai-style custard cake containing different substitution levels of avocado pulp puree

Textural properties	Sample				
	F1	F2	F3	F4	F5
Hardness (g)	123.44 ± 3.42^{c}	125.18 ± 12.46^{bc}	126.89 ± 5.82^{b}	126.89 ± 9.40^{b}	131.17 ± 11.00^{a}
Adhesiveness (g/s)	–46.47 ± 5.69^{a}	–53.19 ± 6.82^{b}	–55.54 ± 6.49^{bc}	–57.04 ± 8.31^{bc}	–59.38 ± 7.00^{c}
Springiness (mm)	0.96 ± 0.06^{a}	0.97 ± 0.02^{a}	0.97 ± 0.03^{a}	0.92 ± 0.09^{b}	0.90 ± 0.06^{b}
Cohesiveness	0.51 ± 0.01^{a}	0.51 ± 0.01^{a}	0.47 ± 0.02^{c}	0.49 ± 0.00^{b}	0.47 ± 0.08^{c}
Chewiness (g)	56.43 ± 19.77^{c}	56.56 ± 7.81^{c}	57.90 ± 4.25^{c}	63.44 ± 6.43^{b}	65.62 ± 6.36^{a}

Mean ± standard deviation (n=3). Values with different superscript letters in a row differ significantly ($P < 0.05$). F1 = control, F2 = 25% APP, F3 = 35% APP, F4 = 45% APP, F5 = 55% APP. APP = avocado pulp puree.

Sensory Analysis of TSC

The data on the sensory scores of the finished products are listed in Table 4. For all the organoleptic properties of the TSC tested by the panelists, no significant differences ($P > 0.05$) were observed in the color and aroma. The mean for the texture for almost all formulations was not significantly different except for the F5 formulation. Regarding taste, the F1 and F2 had the highest score at 7.50 but no significant difference from the F3 and F4 formulations with a verbal interpretation of moderately. The overall acceptance of TSC was greatly influenced by the texture and taste. Thus, applying APP up to 35% substitution resulted in acceptable TSC. In comparison, more than 35% substitution lowered the preference of TSC among panelists, which indicates that higher avocado substitution negatively affected the sensory properties.

The taste and overall acceptability gradually decreased with an increase in avocado pulp puree (APP), which is attributed to the distinctive aftertaste

due to the long-chain C17-aliphatic triols 1,2,4-trihydroxyhepta-deca-16-yne and 1,2,4-trihydroxyheptadeca-16-ene were successfully identified in the skin, seed, and pulp of avocado, exhibiting an unpleasant bitter off-flavor. Moreover, 1-acetoxy-2,4-dihydroxyheptadeca-16-ene, C17-C21oxylipins with 1,2,4-trihydroxy-, 1-acetoxy-2,4-dihydroxy-, and 1-acetoxy-2-hydroxy-4-oxo motifs, 1-O-stearoyl-glycerol and 1-O-linoleoyl-glycerol were reported as bitter-tasting compounds in thermally processed avocado (*Persea americana* Mill.). Unfortunately, Thermal treatment or air-drying of avocado and products made thereof has long been known to induce the development of an unpleasant off-taste centering around a slightly pungent mouthfeel and a pronounced lingering bitter after-taste (Brown, 1972; Degenhardt and Hofmann, 2010). Considering overall acceptability there were no significant differences among the products. So, the highest substitution level is 35 g by APP from 100 g coconut milk in Thai-style custard cake.

Table 4. Influence of avocado pulp puree substitution on sensory qualities of Thai-style custard cake

Textural properties	Sample				
	F1	F2	F3	F4	F5
Color	7.40 ± 1.40	7.40 ± 1.32	7.23 ± 1.35	7.13 ± 1.45	6.96 ± 1.56
Aroma	7.35 ± 1.47	7.16 ± 1.36	7.33 ± 1.34	7.03 ± 1.37	7.13 ± 1.38
Texture	7.26 ± 1.22^{ab}	7.23 ± 1.86^{ab}	7.36 ± 1.21^{ab}	7.30 ± 1.44^{ab}	6.93 ± 1.61^{b}
Taste	7.50 ± 1.26^{ab}	7.50 ± 1.47^{ab}	7.30 ± 1.23^{ab}	7.23 ± 1.65^{ab}	6.80 ± 1.84^{b}
Overall acceptability	7.42 ± 0.97^{ab}	7.30 ± 1.26^{b}	7.40 ± 1.51^{ab}	7.03 ± 1.42^{b}	6.93 ± 1.65^{b}

Mean ± standard deviation (n=3). Values with different superscript letters in a row differ significantly ($P < 0.05$). F1 = control, F2 = 25% APP, F3 = 35% APP, F4 = 45% APP, F5 = 55% APP. APP = avocado pulp puree.

Calorific and nutritional value of TSC: Energy and nutrients

The calorific and nutritional value of Thai-style custard cakes was evaluated by calculating the energy and nutrients per serving for one piece of 104-g Thai-style custard cakes, as summarized in Table 5. The selected formulation (F3) was lower in calorie content, protein carbohydrate, fat, and saturated fatty acid but higher in dietary fiber and unsaturated fatty acid than the control. Protein and fat content were slightly decreased because the protein content in avocado pulp puree was less than in coconut milk. In contrast, the dietary fiber of custard cake was increased from 5.52 to 6.25 g (12.23%) by partially replacing coconut milk with 35% avocado pulp. Avocados are a good source of fiber, low in total carbohydrates, and rich in monounsaturated fats. According to the USDA, one avocado contains around 13.5 grams of fiber, nearly half of the 2020 - 2025 Dietary Guidelines for Americans' recommendation of 28 to 34 g per day (U.S. Department of Agriculture & U.S. Department of Health and Human Services, 2020). Dietary fiber is shown to have numerous benefits, such as improved intestinal function, cholesterol reduction, and increased microbial biomass. The fat content of muffins decreased from 46.06 to 45.68 (0.82%), with the addition of APP in the formula. The saturated fatty acid (SFA) and unsaturated fatty acid (USFA) improved as predicted. The unsaturated fatty acid was increased in Thai-style custard cake incorporating avocado pulp puree (F3) compared to the control. In general, SFA was decreased while USFA was increased with avocado incorporation. The total SFA decreased by 25.29% from the control to the F3 sample. The USFA content, on the other hand, increased by 1.62-fold from the control to the F3 sample. Previous studies also showed a similar trend, in which avocado pulp puree progressively improves the SFA and USFA content in food products (Moolwong et al., 2023; Nurul Ain et al., 2016).

Table 5. Calorific and nutritional value of Thai-style custard cake per piece of 104-g Thai-style custard cake

Calorific and nutritional value	Sample		Nutrient changes
	Control	F3 (35% APP)	
Energy (kcal)	327.58	317.08	–3.21%
Protein (g)	10.99	10.94	–0.41%
Carbohydrate (g)	13.94	12.54	–10.01%
Fat (g)	45.68	46.06	–0.82%
Dietary fiber (g)	5.52	6.20	+12.23%
Saturated fatty acid (mg)	7.63	5.70	–25.29%
Unsaturated fatty acid (mg)	2.59	4.20	+1.62-fold

APP = avocado pulp puree.

CONCLUSIONS

The Thai-style custard cakes incorporating avocado pulp puree were accepted up to 35% avocado incorporation, with 0.82% fat reduction and 3.21% lower calorie content compared to the control sample. Sensorial studies revealed that the acceptability of avocado-incorporated Thai-style custard cakes depends on their texture and taste properties. Therefore, further study must be done to eliminate the aftertaste and unfavorable flavor of avocado pulp puree in another product at full-fat substitution in order to produce a full-fat replacement in the product.

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