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original scientific paper

Antioxidant and Anti-Inflammatory Activities of the Extract and Bioaccessible Fraction of Mango Peel in Muffins

Running head: Antioxidant and Anti-Inflammatory Activities of Mango Peel

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SUMMARY

Research background. Mango peels represent production waste and can cause environmental problems, but they contain nutritive values including bioactive compounds which would be health benefit for human health. The objective of this study was to determine the bioactive compounds, the antioxidant and anti-inflammatory activities of mango peels and their application in muffins.

Experimental approach. A mango peel extract was assessed for its polyphenol, carotenoid, total phenolic contents and antioxidant activity. The anti-inflammatory activity of the extract was investigated using Caco-2 cell assay. Subsequently, mango peel powder was incorporated into muffin formulations. The sensory characteristics of these fortified muffins were assessed. Additionally, total phenolic content, antioxidant activity, and anti-inflammatory properties of the muffin extracts and their bio accessible fractions were analyzed.

Results and conclusions. The mango peels contained quercetin, phenolic compounds, α -carotene, β -carotene and lutein which have antioxidant potential. In Caco-2 cells exposed to induced

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inflammation, the mango peel powder extract (at concentrations of 10, 50, and 100 µg/mL) mitigated the production of reactive oxygen species (ROS), tumor necrosis factor-alpha (TNF-α), and interleukin-8 (IL-8), while simultaneously maintaining cellular viability. Muffins supplemented with 5 % mango peel powder showed good sensory attributes, but lower than standard muffins without mango peel powder. The total phenolic content and antioxidant activities in both the extract and bioaccessible fraction of the supplemented muffins were higher than those observed in the standard muffins. Moreover, the extract and bioaccessible fraction of the supplemented muffins resulted in a greater reduction in ROS, IL-8, and TNF-α production in Caco-2 cells compared to those derived from the standard muffins.

Novelty and scientific contribution. This study is the first to evaluate the protective effects of mango peels and muffins supplemented with mango peel powder against IL-1β-induced oxidative damage in Caco-2 cells. The results confirm that mango peel, as well as the supplemented muffins, inhibited the production of inflammatory markers, including ROS and cytokines. These findings suggest that mango peel could be a valuable ingredient in functional food formulations including dietary supplements products.

Keywords: mango peel; by-products; antioxidant activity; anti-inflammatory activities; well being

INTRODUCTION

Thailand is a major mango-producing country, and mangoes have an important role in the country's economy. Mangoes can be consumed fresh or in processed forms such as juices and purees or canned and dried. The consumption and processing of mango create waste materials, including peels and stones, which are not commonly consumed (1). Mango peels from processing are disposed of as a waste and may cause environmental problems. Numerous studies have shown that mango peels contain nutritive values which can be health benefit for humans, including antioxidants such as carotenoids, polyphenols and flavonoids; carbohydrates; proteins; fats; dietary fibre; calcium; zinc; iron; and manganese (2,3), but they are not regularly consumed. However, most research concentrates on treating waste to extract bioactive compounds, a process that requires additional steps and consequently produces other types of remained waste including chemical waste. Considering these facts, the utilization of mango peels as a functional food ingredient to develop functional foods not only serves to reduce waste but also promotes the sustainable utilization of resources to support food security. Furthermore, it contributes to an enhanced nutritive value and offers human health benefits. Recently, mango by-products have been utilised in baked goods and food products such as pasta, noodles, tortilla chips, bread, biscuits and muffins (4). Besides mango

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peels, other by-products can be added to muffins, including goji berry by-products, grape by-products, pecan nut expeller meal, pomegranate peel and aniseed (5-9). However, research on the biological activities, specifically focusing on the antioxidant and anti-inflammatory properties, of food products integrating by-products such as mango peels, as well as the biological activities of the bioaccessible fractions derived from these products, are still relatively limited.

Therefore, the present study aimed to investigate the proximate composition and bioactive compounds on the antioxidant, the anti-inflammatory properties of mango peel powder and its supplemented food as muffin products. The findings of this study could provide novel insights into utilization of mango peel for health benefit and also lowering waste from mango peel.

MATERIALS AND METHODS

Mango peel preparation

Fah-Lan mangoes (*Mangifera indica*) were collected from a local fruit market in Bangkok, Pathumthani and Suphanburi provinces, Thailand. The mangoes were washed twice with tap water followed by peeling. The mango peels were dried with a towel and subsequently cut into small pieces. These pieces were then subjected to further drying in a hot air oven at 50 °C for 8 h (UF110, Memmert, Düsseldorf, Germany) until their moisture content was reduced to less than 10 % (3,10); note that this drying negatively affects the antioxidant potential (3). The dried mango peel was ground and sifted through a 60 mesh sieve (V8SF, Gilson, Lewis Center, Ohio, USA).

Proximate composition

Standardized methods from the Association of Official Analytical Chemists (AOAC) (11) were employed to analyze the moisture, crude protein, crude fat, crude fibre, total sugar, and ash content in the dried mango peel powder. The carbohydrate content (in %) was determined using the difference method, which calculates the remaining macronutrients after accounting for moisture, proteins, fats, and ash.

$$w(\text{carbohydrate}) = (100 - w(\text{moisture}) - w(\text{protein}) - w(\text{fat}) - w(\text{ash})) / 11$$

Determination of phenolic compounds

Briefly, sample (10 g) was added to aqueous methanol (62.5 %, 40 mL) containing 0.5 g/L *tert*-butylhydroquinone (Sigma-Aldrich, St. Louis, MO, USA) and 10 mL of 6 N HCl (Merck, Darmstadt, Germany). Following by incubation of the sample in water bath shaker (at 90 °C for 2 h) (Mettler, Germany) and then cooled at room temperature. Subsequently, ascorbic acid (Ajax Finechem, Victoria, Australia) was added (100 µL of 1 %), and each sample was diluted to 50 mL with methanol

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(Merck). The solution was subjected to sonication (5 min) followed by filtration through a 0.2 μm polytetrafluoroethylene (PTFE) syringe filter (Chrom Tech, Milford, MA, USA). The solution was analyzed by high-performance liquid chromatography (HPLC) to quantify the following phenolic compounds: p-coumaric acid, caffeic acid, ferulic acid, sinapic acid, apigenin, hesperetin, kaemferol, luteolin, myricetin, naringenin, naringin, limonin, and quercetin (Sigma-Aldrich). The HPLC system utilized included a quaternary gradient pump (G1315A), a vacuum degasser (G1379A), and a Zorbax Eclipse XDB-C18 column (250 \times 4.6 mm, 5 μm) with a matching guard column (Agilent Technologies, Santa Clara, CA, USA), maintained at 30 °C. Detection was conducted using a diode array detector (G1315B) across wavelengths of 210, 280, 325, 338, and 368 nm. The flow rate was adjusted to 0.6 mL/min with water (A), methanol (B), and acetonitrile (C), each containing 0.05 % trifluoroacetic acid. The solvent gradient was programmed as follows: 0–5 min (90–85 % A, 6–9 % B, 4–6 % C), 5–30 min (85–71 % A, 9–17.4 % B, 6–11.6 % C), 30–60 min (71–0 % A, 17.4–85 % B, 11.6–15 % C), 60–61 min (0–90 % A, 85–6 % B, 15–4 % C), and 61–66 min (90 % A, 6 % B, 4 % C) (12). Recovery rates for each standard ranged from 97 to 103 %, with results expressed as mg/100 g of sample.

Determination of carotenoids

Briefly, sample (10 g) was transferred into a brown round-bottom flask. Then, ascorbic acid (10 mL of 10 %) (Ajax Finechem, NSW, Australia) and ethanolic potassium hydroxide (KOH) (50 mL of 2M) (Ajax Finechem, NSW, Australia) were added. This solution was placed in a boiling water bath for 30 min and then cooled at room temperature. The sample was extracted with 70 mL of hexane (J.T. Baker, Phillipsburg, NJ, USA). Following phase separation, the upper layer was decanted into an amber glass separating funnel containing 50 mL of a 5 % (*m/V*) potassium hydroxide (KOH) solution. It was shaken and the lower solution was discarded. The sample was rinsed with 100 mL of water until it was devoid of alkalis. A portion of the solution was collected and subjected to evaporation using a rotary evaporator (Buchi, Flawil, Switzerland) under vacuum at 40 °C. The sample residue was dissolved in methylene chloride (CH_2Cl_2 : J.T. Baker, Phillipsburg, NJ, USA) and mobile phase (1:2) before analysis. Carotenoid compounds, comprising α -carotene, β -carotene, β -cryptoxanthin, lutein, lycopene, and zeaxanthin, were quantified using an Agilent 1260 Infinity system equipped with an ultraviolet/visible (UV/VIS) detector manufactured by Agilent Technologies. The separation of carotenoids was achieved using a C18 column (4.6 \times 250 mm, 5 μm ; Vydac 201TP, Grace Davison, Waltham, MA, USA) equipped with a guard column (4.6 \times 12.5 mm, 5 μm ; Vydac 201TP, Grace Division). The separation was performed at a flow rate of 0.7 mL/min at 30 °C, with monitoring conducted at 450 nm. The mobile phase was a mixture of HPLC-grade acetonitrile, methanol,

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methylene chloride, triethylamine and ammonium acetate (90:8:2:0.085:0.085, by volume) (12). For each standard compound, the recovery percentage was 96–102 %. The carotenoid content is reported as mg/100 g of sample.

Evaluation of the total phenolic content and antioxidant activity

Sample extraction

Sample was extracted using 90 % ethanol at a 1:10 (*m/V*) ratio and sonicated for 10 min in a sonicator bath (Mettler Electronics Corp, Anaheim, CA, USA). Following sonication, the mixture was centrifuged (Becton Dickinson Dynac Centrifuge, Sparks, MD, USA) at 5 600×g for 10 min at 25 °C. The supernatant was filtered and collected, while the residue underwent two additional extractions, and the combined filtrates were pooled.

The supernatant was filtered and collected, whereas the residue was extracted twice with the same extract solution. The filtrates were combined and concentrated using a rotary evaporator under vacuum at 40 °C. Subsequently, the concentrated extract was dissolved again in deionized water for analysis (13).

Evaluation of the total phenolic content

In brief, 0.5 mL of extract, 8.0 mL of Folin-Ciocalteu reagent, 0.5 mL of distilled water and 1 mL 20 % of Na₂CO₃ were mixed and incubated in the dark (14). The measurement of absorbance at 750 nm was conducted using a UV/VIS spectrophotometer (model UV-1601, Shimadzu, Chiyoda-ku, Tokyo, Japan). The total phenolic content was determined by using a gallic acid calibration curve and is presented as mg gallic acid equivalents (GAE)/g.

Evaluation of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

Twenty-two microliters of either sample extract or Trolox were combined with 200 µL of 150 µM DPPH and incubated in the dark room for 30 min (15). The absorbance at 517 nm was assessed using a microplate reader (Sunrise, Tecan Co., Grödi, Austria). Antioxidant activity was determined utilizing a Trolox calibration curve and is expressed as µmol Trolox equivalents (TE)/g.

Evaluation of the ferric reducing antioxidant power (FRAP)

Twenty microliters of either sample extract or ferrous sulphate heptahydrate (FeSO₄·7H₂O) were combined with 150 µL of FRAP reagent and incubated in the dark room for 8 min (16). The absorbance at 600 nm was then measured using a microplate reader (Sunrise, Tecan Co., Austria). The antioxidant activity is reported as µmol TE/g.

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Determination of the oxygen radical absorbance capacity (ORAC)

Five hundred microlitres of sample extract or 6.25-100 μmol Trolox was mixed with 3 mL of 4.19 μM fluorescein. The solution was incubated at ambient temperature for 8 min, then 0.5 mL of 153 mM 2,2-azobis [2-amidino-propane] dihydrochloride (AAPH) was added (17). Absorbance was assessed by luminescence spectrofluorometer (model LS 55, Perkin Elmer, Waltham, MA, USA) at an excitation wavelength of 495 nm and an emission wavelength of 515 nm. The antioxidant activity is presented as $\mu\text{mol TE/g}$. A 75 mM phosphate buffer was used as a blank (control).

Determination of anti-inflammatory activity using Caco-2 cells

Cell culture

Caco-2 cells were obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA) and cultured in 6-well plates using Dulbecco's Modified Eagle's Medium (DMEM, Sigma-Aldrich) supplemented with 15 % heat-inactivated fetal bovine serum (FBS, Sigma-Aldrich), 1 % nonessential amino acids, 1 % L-glutamine, 0.2 % fungizone, and 1 % penicillin-streptomycin (Gibco, Leicestershire, UK) within a controlled environment (95 % air and 5 % CO_2 , V/V) at 37 °C. Medium renewal occurred every 2 days, and the FBS concentration was reduced to 7.5 after 4–5 days of culture. Experimental procedures were conducted using cultures aged between 11 and 14 days (18).

Cytotoxicity test

The cytotoxicity test was conducted according to the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide tetrazolium (MTT) assay. Caco-2 cells were washed with serum-free medium before using. Subsequently, Caco-2 cells were exposed to mango peel extract with varying concentrations (0–200 $\mu\text{g/mL}$) at 37 °C for 24 h. Following this incubation period, the cells were washed with phosphate-buffered saline (PBS), followed by the addition of 2.0 mL of MTT solution (0.5 mg/mL in PBS). The plate was further incubated at 37 °C for 4 h. Post-incubation, the MTT solution was aspirated, and the formazan crystals produced by viable cells were dissolved with dimethyl sulfoxide (DMSO). Absorbance measurements were conducted at 540 nm using a microplate reader (SPECTROstarNano, BMG LABTECH, Offenburg, Germany). Furthermore, if absorbance values exceeded 90 % of the control samples, the results were corrected (19).

Determination of anti-inflammatory activity

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Caco-2 cells were cultured with mango peel powder extract in a controlled environment (95 % air and 5 % CO₂, V/V) at 37 °C for 1 h. Subsequently, inflammation was induced by exposing the cells to 10 ng/mL interleukin-1 β (IL-1 β) at 37 °C for 30 min. The cells were harvested for the evaluation of reactive oxygen species (ROS) production using 2',7'-dichloro-fluorescein diacetate (DCFH-DA) (20). Additionally, the culture medium was collected to quantify tumor necrosis factor-alpha (TNF- α) and interleukin-8 (IL-8), levels using enzyme-linked immunosorbent assays (21).

Evaluation the characteristics of muffins supplemented with mango peel powder

Muffin production

The muffins were prepared with cake flour (200 g, UFM, Samut Prakan, Thailand), sodium bicarbonate (4 g, McGarrett, Bangkok, Thailand), double-acting baking powder (8 g, McGarrett, Bangkok, Thailand), sour cream (40 g, Alli, Nakhon Ratchasima, Thailand), salt (2 g, Prungtip, Nakornratchasima, Thailand), pure vanilla extract (2.5 g, McCormick, Hunt Valley, MD, USA), egg (65 g, CP, Bangkok, Thailand), milk (125 g, CP-Meiji, Saraburi, Thailand), butter (50 g, Allowrie, KCG, Bangkok, Thailand) and granulated sugar (140 g, Mitrphol, Lei, Thailand). The egg was beaten before the milk, butter and sugar were added. After obtaining a smooth batter, the remaining dried ingredients were added and mixed. The muffins were baked at 180 °C for 20–25 min. Variants of the muffins were prepared with supplementation levels of 5, 10 or 15 % mango peel powder, which replaced a portion of the total flour.

The muffins were subjected to sensory evaluation using a 9-point hedonic scale, while determination of their colour, firmness, and proximate composition were conducted.

The sensory evaluation engaged 60 untrained panellists, comprising 30 men and 30 women, aged between 18 and 30 years, who possessed prior familiar with muffins. Each sample was assigned a three-digit code. Each panellist received four samples with water to cleanse the palate between the samples. A 9-point hedonic scale ranging from 1 (representing "extremely dislike") to 9 (representing "extremely like") was employed for assessing the overall acceptability.

The colour of the muffin was assessed in triplicate by using a colorimeter JWF30, Fru, Shenzhen, China(. Briefly, the colorimeter was placed on the surface of muffin to measure the L^* (lightness), a^* (redness), and b^* (yellowness) values.

The muffin texture, including hardness, springiness, cohesiveness, and chewiness was assessed with a texture analyser (TA.XT2i, Stable Micro Systems, Surrey, UK). Each sample was assessed with a cylindrical shape probe (diameter 100 mm) in three different areas close to the centre. The muffin was cut into cylinders with a diameter of 2.65 cm diameter and a height of 1 cm. The testing speed was set at 1 mm/s, and the strain applied was 25 % of the total height (22).

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The nutritional value of selected muffin was determined by proximate analysis (11). The energy content was determined based on the components that provide energy (carbohydrates, proteins and fats).

Quantification of total phenolic content, antioxidant, and anti-inflammatory properties of the extract and bioaccessible fraction of muffins enriched with mango peel powder

Muffins supplementation with mango peel powder extraction

An extract of a muffin supplemented with mango peel powder was prepared according to Muangnoi *et al.* (13). The quantification of total phenolic content, antioxidant capacity, and anti-inflammatory properties were conducted as described above.

In vitro digestion of muffins supplement with mango peel powder

The muffin (1 g) was mixed with 30 mL of 120 mM NaCl and then subjected to simulated gastric and small intestinal digestion according to Dawilai *et al.* (23) and Ferruzzi *et al.* (24). The pH of the sample solution was adjusted to 2.0 with 1 M HCl. Subsequently, the samples were digested with 2 mL of pepsin in a shaking water bath at 37 °C for 1 h. The pH was adjusted to a range of 6.5 to 6.8 using 1 M sodium bicarbonate (NaHCO₃) before the addition of 3 mL of bile extract along with 2 mL of pancreatin and lipase. The samples were shaken in a shaking water bath at 37 °C for 2 h. The digested (bioaccessible) fraction was separated by centrifugation at 10 000×g for 1 h and subsequently filtered through 0.2-µm filter paper. The obtained sample was purged with nitrogen and stored at -80 °C until analysis.

Statistical analysis

The data were collected in triplicate and subjected to statistical analysis to determine significance using *t*-test and ANOVA (25). Duncan's new multiple range test was utilized to evaluate distinctions among group means. Results are expressed as mean±standard deviation (S.D.), with all assays conducted in triplicate for each independent treatment.

RESULTS AND DISCUSSION

Proximate composition, active substances and antioxidant potential of mango peel powder

Table 1 presents the proximate composition of the mango peel powder. It contained 84.78 % carbohydrates, of which 43.17 % was fibre. The findings corresponded closely with those reported in

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the investigations conducted by Ajila *et al.* (10) and El-Faham *et al.* (26), who reported that carbohydrates, including crude fibre, were the dominant component of mango peel powder.

Of the phenolic compounds, only quercetin was detected while other phenolic compounds were not detected due to laboratory limitations. In contrast with previous report, ferulic acid and caffeic acid have been identified in raw Fah-Lan mango peel (27). The total phenolic content of the mango peel extract for the present study was lower compared to the data documented by Ajila *et al.* (10) and Pinsirodom *et al.* (27) (9.59–9.86 mg GAE/g fresh mass).

There were three carotenoids namely α -carotene, β -carotene and lutein, detected in the mango peel powder extract, β -cryptoxanthin, lycopene and zeaxanthin were not detected. The finding aligned with Ranganath *et al.* (28), who observed that β -carotene, lutein and α -carotene are the dominant carotenoids in mango peel powder extracts from a wide range of cultivars (Hamle, Arka Anmol, Peach, BGNP Banganapalli, Janardhan Pasand, Lalmuni, Gulabi, Bombay No. 1, Lazzat Baksh and Tommy Atkins).

Table 1 presents the antioxidant potential of the powder from mango peel based on the DPPH, FRAP and ORAC assays. The antioxidant activity of mango peels from Sri Lanka showed a higher DPPH value (11.86–18.91 $\mu\text{mol TE/g}$) than this study. In contrast, the FRAP value was lower (23.61–88.31 $\mu\text{mol TE/g}$) (29). Quercetin, lutein, and β -carotene are widely recognized for their potent antioxidant properties attributed to their capacity to neutralize free radicals through electron or hydrogen donation mechanisms (30).

The differences in the proximate composition, active substances and antioxidant potential of mango peels might be due to differences in the mango variety, the growing conditions (including fertilisation), climate, sample collection and preparation (27). Our data indicate that mango peels contain fibre, polyphenols and carotenoids, and they have antioxidant potential. Therefore, mango peels could be used as a supplement in healthy foods.

The effect of mango peel powder extract on Caco-2 viability

Concentrations of 150 and 200 $\mu\text{g/mL}$ were found to be toxic to Caco-2 cells, as indicated by cell viability dropping below 90 %; however, the other concentrations did not exhibit toxicity (**Table 2**). Therefore, the concentrations chosen for the subsequent experiments were 10, 50 and 100 $\mu\text{g/mL}$.

Anti-inflammatory activity of the mango peel powder extract

Fig. 1a, **Fig. 1b** and **Fig. 1c** show the anti-inflammatory activity of the mango peel powder in Caco-2 cells. These cells were pretreated with mango peel powder extract (10, 50 and 100 $\mu\text{g/mL}$) for 2 h before stimulation with 10 ng/mL IL-1 β for 30 min. This pretreatment exhibited a dose-

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dependent reduction in ROS, TNF- α and IL-8 production when compared with Caco-2 cells treated solely with IL-1 β , without impacting cell viability (data not shown). Exposure of Caco-2 cells to mango peel powder extract (10, 50 and 100 $\mu\text{g/mL}$) significantly inhibited IL-8 secretion (6.35, 27.47 and 61.56 %, respectively); the concentrations of 50 and 100 $\mu\text{g/mL}$ significantly inhibited ROS (28.02 and 61.91 %, respectively) and TNF- α (29.31 and 65.93 %, respectively) production. These data indicate that the mango peel powder extract has anti-inflammatory activity, perhaps because it contains quercetin, lutein and β -carotene, which have an anti-inflammatory ability. Previous studies have shown that pretreatment of PC-12 cells with quercetin significantly reduced intracellular ROS production when exposed to hydrogen peroxide (H_2O_2) (31). Kumar *et al.* (32) documented that diabetic retinas treated with quercetin exhibited a notable reduction in TNF- α expression compared to untreated diabetic retinas. Neelam *et al.* (33) revealed that lutein administration decreased serum ROS levels in patients with non-proliferative diabetic retinopathy. In addition, lutein demonstrated dose-dependent reduction in lipopolysaccharide-induced IL-8 secretion in uveal melanocytes (UM) cells (34). Moreover, Takahashi *et al.* (35) found that oral administration of β -carotene to mice reduced inflammation and irritative effects associated with atopic dermatitis by suppressing the expression of TNF- α .

Sensory and physical properties of muffins supplemented with mango peel powder

Table 3 shows the effect of supplementing muffins with mango peel powder on their sensory and physical properties. For the sensory evaluation, the standard muffins without mango peel powder obtained the highest scores for all characteristics. The addition of mango peel powder resulted in a significant reduction in sensory attributes ($p < 0.05$). Among the supplemented muffins, those with 5 % mango peel powder had the highest sensory scores.

The amount of mango peel powder added to the muffins significantly affected the lightness and yellowness of the muffins. Similarly, the biscuits became darker as the amount of mango peel powder they contained increased (10). Mango peel powder has a brown colour due to enzymatic browning reaction occurring during mango peel powder processing, so adding it to muffins decreases the lightness of the product. Moreover, lightness of cakes decreased with an increasing amount of potato peels (36). The b^* indicates the yellowness; it increased gradually as the amount of mango peel powder increased. There was no significant difference for redness.

The mango peel powder also affected the textural characteristics of the muffins. Specifically, the hardness of the muffins increased as the amount of mango peel powder increased due to the high fibre content of this powder. Vice versa, as the amount of mango peel powder increased, the springiness, and cohesiveness of the muffins decreased. The findings are consistent with the results

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demonstrated by Nakov *et al.* (37). The muffins with 5 % mango peel powder showed significantly different hardness and chewiness compared with the standard muffins. Thus, the most appropriate mango peel supplementation is 5 %, which represents approximately 1.5 % of the total muffin mass.

The muffins were also subjected to proximate analysis. The muffins had similar fat and ash contents, but the crude fibre of muffins containing 5 % mango powder peel was higher than the standard muffins (Table 4). Consistently, many researchers have found that by raising the percentage of fruit by-products such as apple pomace (38), mango peel powder (39) and rape pomace (37), the total dietary fibre content in baked goods increases. The results indicate that mango peels can be an alternative source of fibre in baked goods.

The total phenolic content and antioxidant potential of standard muffins and muffin containing mango peel powder

Fig. 2 demonstrates the total phenolic compounds and antioxidant potential of the extracts and bioaccessible fractions of standard muffins and muffins supplemented with 5 % mango peel powder. The total phenolic content in the extract and bioaccessible fraction of the supplemented muffins ((15.91±0.19) and (9.58±0.15) mg GAE/g, respectively) was significantly higher than the content of the standard muffins ((8.01±0.16) and (6.78±0.12) mg GAE/g, respectively) ($p < 0.05$).

The DPPH, FRAP and ORAC values were (0.14±0.01), (80.93±4.21) and (0.96±0.02) $\mu\text{mol TE/g}$, respectively, for the extract of muffins supplemented with 5 % mango peel powder, and (0.13±0.01), (62.54±1.30) and (0.65±0.04) $\mu\text{mol TE/g}$, respectively, for the extract of standard muffins. The extract of the supplemented muffins had significant FRAP and ORAC values than the of extract standard muffins; there were no significant differences observed between the extracts of supplemented and standard muffins regarding DPPH values ($p > 0.05$).

The bioaccessible fraction of muffins supplemented with 5 % mango powder peel had significantly higher antioxidant activity ((0.04±0.01), (9.10±0.32) and (2.88±0.07) $\mu\text{mol TE/g}$ for DPPH, FRAP and ORAC assay, respectively) compared with the bioaccessible fraction of standard muffins ((0.01±0.01), (6.62±0.97) and (1.98±0.12) $\mu\text{mol TE/g}$, respectively) ($p < 0.05$). The total phenolic content of the bioaccessible fractions were lower than the total phenolic content of the extracts. Researchers have reported a lower total phenolic content after *in vitro* digestion of samples such as apples, pineapples, mangoes and papayas (40) as well as apples and enriched apple snacks with grape juice and coffee pulp (41) compared with undigested samples. This might due to interactions between phenolic compounds and digestive enzymes and other components such as buffers and electrolytes (40) or instability of quercetin during digestive condition (42).

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The DPPH and FRAP values for the bioaccessible fractions were lower than the values for the extracts. Velderrain-Rodríguez *et al.* (40) and Khochapong *et al.* (41) reported comparable findings. However, the ORAC values of the bioaccessible fractions were higher than the values of the muffin extracts. Yida *et al.* (43) reported that at similar concentrations, digested edible birds' nest had increased ORAC activity while undigested edible birds' nest had little ORAC activity. Viduranga (44) also revealed that bioaccessible fractions of *Pouteria campechiana* pulp had higher ORAC activity than undigested sample, whereas the polyphenol content and DPPH activity were lower than the values in undigested samples.

Anti-inflammatory activity of muffin extracts and bioaccessible fractions

The anti-inflammatory activity results are expressed as the percentage inhibition of inflammatory marker production (Fig. 3). Caco-2 cells were pretreated with muffin extracts (10, 50 and 100 µg/mL) or bioaccessible fractions for 2 h and then stimulated with 10 ng/mL IL-1β for 30 min. The standard muffin extract (10, 50 and 100 µg/mL) or supplemented muffin extract (10, 50 and 100 µg/mL) significantly reduced ROS production (1.19–4.88 and 2.58–8.33 %, respectively), IL-8 production (3.08–6.08 % and 3.65–9.23 %, respectively) and TNF-α production (3.12–9.95 % and 3.18–14.03 %, respectively). The bioaccessible fraction of standard muffins decreased ROS, IL-8 and TNF-α production by 2.74, 1.47 and 3.74 %, respectively. The bioaccessible fraction of supplemented muffins suppressed ROS, IL-8 and TNF-α production by 8.48, 2.34 and 9.09 %, respectively. The suppressed inflammatory marker production might be due to muffin ingredients such as egg, fresh milk and butter. Researches have reported that whole egg consumption reduced TNF-α production in people with metabolic syndrome (45) and type 2 diabetes (46). Phosphatidylcholine is the predominate phospholipid found in eggs; it reduced the lipopolysaccharide-induced TNF-α secretion in a rodent model of systemic inflammation (47). Pasteurised cow's milk reduced the expression of inflammatory cytokines such as IL-1, IL-6, and IL-8 in human gingival fibroblasts and human oral epithelial cell line HSC2 (48). Nestel *et al.* (49) showed that overweight adults who consumed butter had reduced secretion of inflammatory markers (IL-6, TNF-α and IL-1β).

Overall, the muffins supplemented with mango peel powder had better anti-inflammatory activity than the standard muffins, perhaps due to the quercetin, lutein and β-carotene content in mango peels.

CONCLUSIONS

Mango peels contain many nutrients and beneficial substances, such as dietary fibre, carotenoids and polyphenols, which have antioxidant and anti-inflammatory activities. Moreover,

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mango peel extract has the potential to suppresses the production of inflammatory markers such as ROS, TNF- α and IL-8. Muffins containing 5 % mango peel showed high consumer satisfaction scores, although they were still lower than standard muffins. These muffins contained three times as much fibre as standard muffins. In addition, their extract and bioaccessible fraction exerted greater antioxidant potential and anti-inflammatory activity of gut cells compared with standard muffins.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

Yossaporn Plaitho contributed to the study design, collected the data, analysed and interpreted the data, writing and revising the manuscript. Aikkarach Kettawan contributed to the study design, including data analysis, interpretation, suggestion, conclusion, critical revision of the overall results, and revising the manuscript. Hataichanok Sriprapai contributed to design and implementation of the muffin-related research, including data analysis and interpretation. Aurawan Kringkasemsee Kettawan contributed to the implementation of the cytotoxicity experiments, including data analysis and interpretation, and critical revision the overall results. Phakpoom Kooprasertying contributed to the data analysis, drafted the article, writing and revising the manuscript. All authors have approved the final version of the manuscript.

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Table 1. The proximate composition, active substances and antioxidant activity of mango peel powder extracts

Component	Content
<i>w</i> /%	
Moisture	6.18±0.26
Carbohydrates	84.78±0.36
Crude fibre as part of carbohydrates	43.17±0.83
Total sugar as part of carbohydrates	12.99±0.32
Protein	3.95±0.12
Fat	1.17±0.09
Ash	3.91±0.25
<i>w</i> (phenolic compounds)/(mg/100 g)	
Quercetin	6.02±0.94
apigenin, caffeic acid, ferulic acid, hesperetin, kaemferol, limonin, luteolin, myricetin, naringenin, naringin, <i>p</i> -coumaric acid, sinapic acid	ND
Total phenolic compounds (mg GAE/g)	6.16±0.19
Carotenoid <i>w</i> (carotenoid)/(mg/100 g)	
α-carotene	0.05±0.001
β-carotene	2.28±0.91
Lutein	2.22±0.12
β-cryptoxanthin, lycopene, zeaxanthin	ND
Antioxidant activity TE/(μmol/g)	
DPPH	3.72±0.01
FRAP	196.34±1.86
ORAC	85.67±1.13

The data are presented as the mean±standard deviation (*N*=3). ND=not detected

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Table 2. Cytotoxicity of the mango peel powder extract

γ (mango peel powder extract)/($\mu\text{g/mL}$)	Cell viability/%
0	(100 \pm 0)
10	(98.70 \pm 3.13) ^a
50	(98.25 \pm 2.70) ^a
100	(96.84 \pm 3.38) ^a
150	(86.43 \pm 0.86) ^b
200	(85.74 \pm 2.20) ^b

The data are presented as the mean \pm standard deviation ($N=3$). Different superscript letters indicate a significant difference in the same column (analysis of variance followed by Duncan's test, $p<0.05$)

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Table 3. Sensory evaluation using a 9-point hedonic scale, and colour and texture analysis of standard muffins and muffins supplemented with different percentages of mango peel powder

Parameter	Score			
	Standard muffin	Percentage of mango peel powder		
		5	10	15
Sensory attribute				
Appearance	(7.78±0.42) ^a	(7.12±0.83) ^b	(6.55±0.57) ^c	(6.10±0.77) ^d
Colour	(7.73±0.80) ^a	(7.20±0.90) ^b	(6.28±0.69) ^c	(6.17±0.72) ^c
Odor	(7.50±0.89) ^a	(7.15±0.82) ^b	(6.63±0.58) ^c	(6.12±0.78) ^d
Texture	(7.55±0.77) ^a	(7.12±0.80) ^b	(6.48±0.65) ^c	(6.22±0.69) ^c
Taste	(7.62±0.78) ^a	(7.17±0.72) ^b	(6.70±0.46) ^c	(6.12±0.78) ^d
Overall acceptance	(7.60±0.85) ^a	(7.15±0.61) ^b	(6.40±0.81) ^c	(6.15±0.40) ^c
Colour				
<i>L</i> [*]	(60.83±0.65) ^a	(43.20±2.86) ^b	(34.36±0.72) ^c	(30.03±2.23) ^d
<i>a</i> ^{*ns}	(6.00±2.12)	(7.96±0.56)	(8.66±1.00)	(9.46±4.35)
<i>b</i> [*]	(12.06±3.52) ^c	(13.77±3.55) ^{bc}	(18.13±0.80) ^b	(23.03±0.40) ^a
Texture				
Hardness	(388.21±6.90) ^d	(459.69±1.98) ^c	(545.90±3.27) ^b	(576.93±2.03) ^a
Springiness	(63.22±3.63) ^a	(57.13±3.51) ^{ab}	(57.66±2.09) ^{ab}	(55.89±3.63) ^b
Cohesiveness	(51.97±2.83) ^a	(50.03±0.98) ^a	(47.85±1.09) ^{ab}	(43.86±3.72) ^b
Chewiness	(148.82±2.84) ^b	(171.50±6.05) ^a	(155.81±6.77) ^b	(136.56±5.81) ^c

The data are presented as the mean±standard deviation (*N*=3). Different superscript letters indicate a significant difference in the same row (analysis of variance followed by Duncan's test, *p*<0.05). ^{ns}indicate no significant difference

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Table 4. Nutritional value of standard muffins and muffins supplement with 5 % mango peel powder (100 g)

Nutrient	Muffin		p
	Standard	Supplement with mango peel	
<i>m</i> (carbohydrate)/g	56.72±0.29	55.89±0.10	0.010
<i>m</i> (crude fibre)/g	0.25±0.03	0.81±0.02	0.000
<i>m</i> (protein)/g	5.75±0.03	5.49±0.01	0.000
<i>m</i> (fat)/g	10.96±0.20	10.72±0.13	0.165
<i>m</i> (moisture)/g	24.32±0.18	25.28±0.22	0.004
<i>m</i> (ash)/g	2.01±0.11	1.80±0.14	0.110
<i>E</i> /kJ	1458.07±4.56	1431.09±6.39	0.004

The values are presented as the mean±standard deviation ($N=3$). The data were compared with an independent *t*-test ($p<0.05$)

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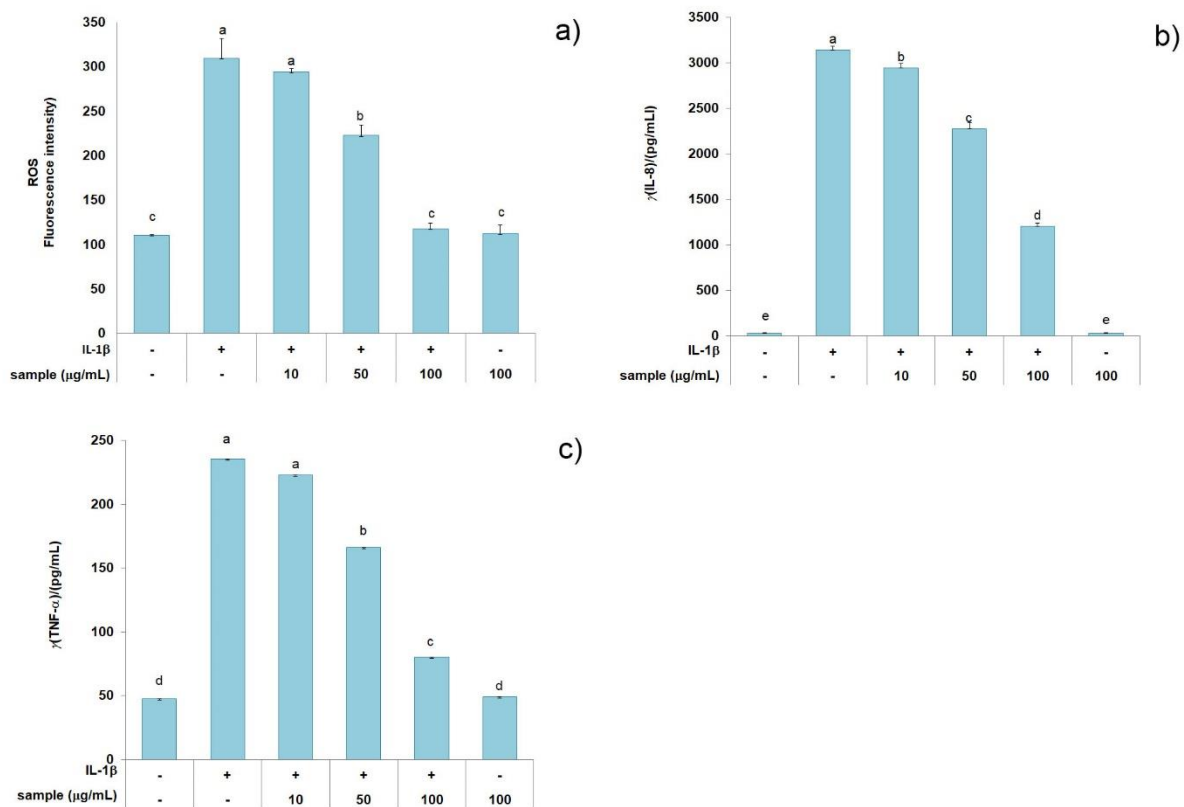


Fig. 1. Suppression of IL-1 β -induced (a) ROS, (b) IL-8 and (c) TNF- α production by mango peel powder extract. Different letters indicate a significant difference ($p < 0.05$)

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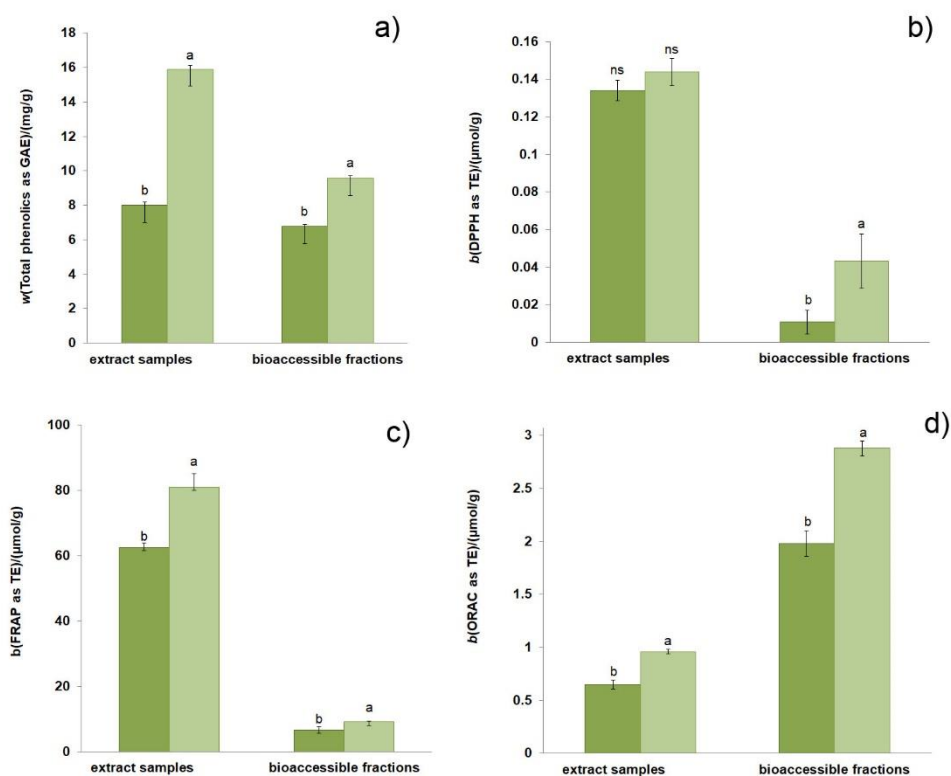


Fig. 2. The (a) total phenolic content and (b) DPPH, (c) FRAP and (d) ORAC activities of the extracts and bioaccessible fractions of standard muffins (■) and muffin supplement with 5 % mango peel powder (■). Different letters indicate a significant difference ($p < 0.05$)

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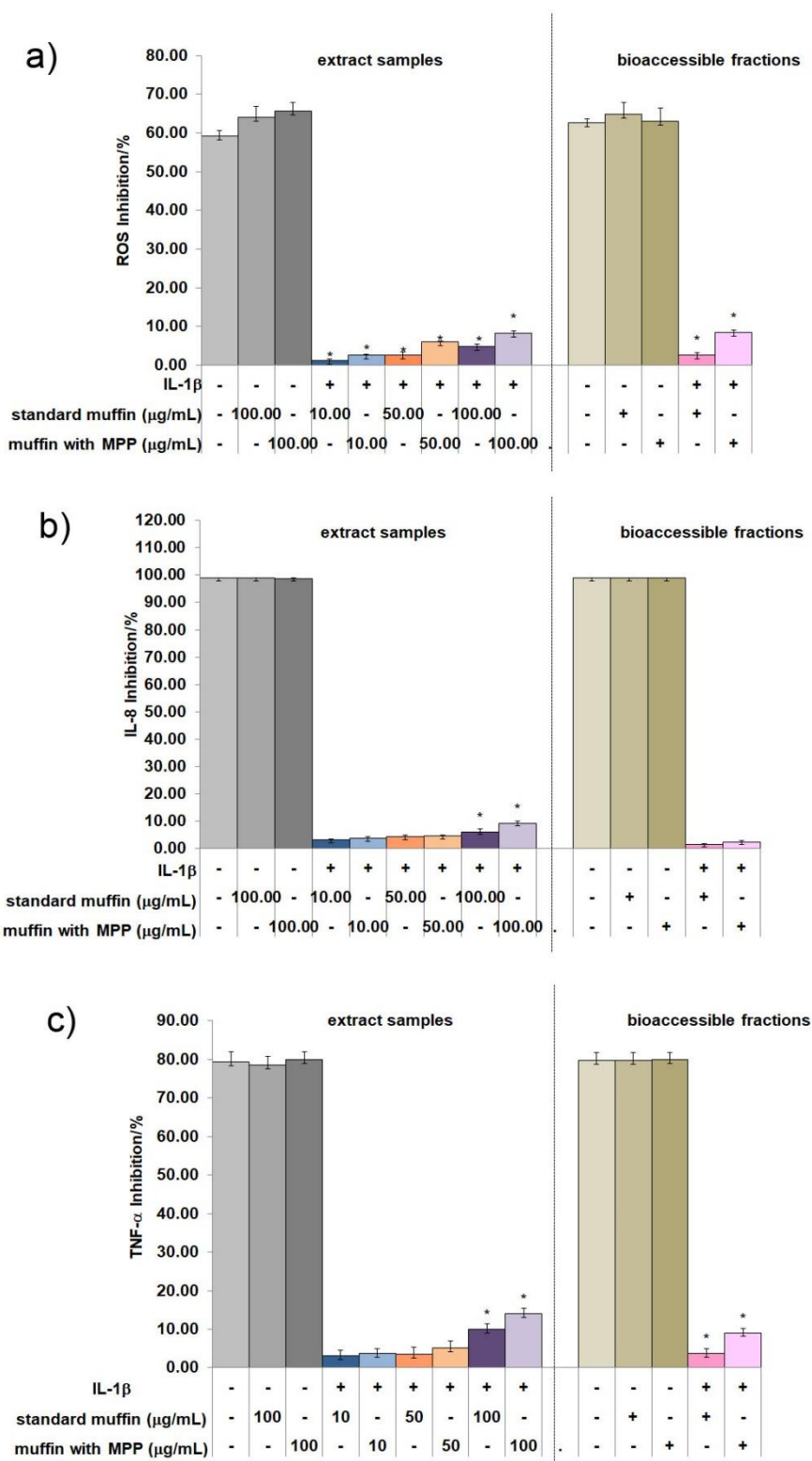


Fig. 3. Percentage inhibition of the extracts and bioaccessible fractions of standard muffins and muffins supplemented with 5 % mango peel powder of IL-1 β -induced (a) ROS, (b) IL-8 and (c)TNF- α production.

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*indicates a significant difference ($p < 0.05$) between standard muffins and muffin supplement with mango peel powder at the same concentration