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Research Article

Digestive Enzyme Inhibition of Different Phenolic Fractions and Main Phenolic Compounds of Ultra-High-Pressure-Treated Palm Fruits: Interaction and Molecular Docking Analyses

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The purpose of the present work was to evaluate the inhibitory effects of different phenolic extracts from non- and ultra-high-pressure- (UHP-) treated palm fruits and their main phenolic compounds against pancreatic lipase and α-glucosidase and to further analyze the interaction and inhibitory mechanisms of two main phenolics (caffeic acid and catechin). Results showed that the free, esterified, and insoluble-bound phenolic fractions from the non- and UHP-treated fruits demonstrated good inhibitory effects towards two enzymes. The insoluble-bound phenolic fraction, regardless of UHP treatment, presented the strongest inhibitory capacities, and UHP treatment significantly upgraded the inhibitory effects of these phenolic fractions (lipase IC_{50}: 78.01 vs. 72.50 μg/mL; α-glucosidase IC_{50}: 76.42 vs. 64.51 μg/mL). Catechin and caffeic acid, main phenolic compounds detected in all phenolic fractions of the fruits, showed similar efficiencies on inhibiting the two enzymes, which were consistent with the findings observed by molecular docking analysis. Moreover, these two phenolic compounds exhibited a synergy effect on inhibiting pancreatic lipase and α-glucosidase at a relatively high combination concentration with the ratio of 1 : 1. Therefore, the present work may be helpful for further application of palm fruits as food supplements or nutraceuticals to control energy intake to improving some chronic metabolic diseases.

1. Introduction

With the continuous improvement of people's material life, long-term consumption high-glucose and high-fat diet results in glucose and lipid metabolic disorders, causing severe problems to human health [1]. Glucose and lipid metabolic disorders, a cluster of metabolic syndrome, are closely related with many chronic disease occurrences, such as obesity and diabetes [2]. An epidemiological survey suggested that more than three million people bear the obesity torture in the USA, and about one billion people either being overweight or obesity worldwide [3]. Diabetes (the fifth health killer) threatens the human health by damaging the blood vessels, nerves, and organs [4]. The most common drug to control or improve obesity and diabetes available on the market are orlistat and acarbose. Orlistat can reduce the lipid absorption by inhibiting the lipase activity [5], and acarbose controls postprandial blood glucose by reducing the glucose absorption with inhibiting the α-glucosidase activity [6]. Nevertheless, some unpleasant effect may take place, such as abdominal discomfort and flatulence [5, 6]. Therefore, excavating natural inhibitors of pancreatic lipase and α-glucosidase to control obesity and diabetes is the research hotspots. Many studies have proved that phenolic compounds possess inhibitory effect on digestive enzymes, such as pancreatic lipase and α-glucosidase [7–9]. Meanwhile, phenolic compounds could also be absorbed into human body to directly exert their health benefits [10].

Oil palm (*Elaeis guineensis* Jacq.) fruits, a common economic oil crop for pressing oil [11], are not only abundant with oil but also rich in phenolic compounds [12]. However, to the best of our knowledge, few studies have been performed to evaluate the inhibitory effects of the free, esterified and insoluble-bound phenolic fractions (F, *E*, and IB) from oil palm fruits against the pancreatic lipase and α-glucosidase. Meanwhile, ultra-high-pressure (UHP) treatment, one of the newest food processing methods, has been widely used in food processing industry. UHP greatly improved bioaccessibility and bioactivity of the bioactive compounds in food materials [13]. In our previous study, we found that the phenolic and flavonoid contents of the F, *E*, and IB phenolic fractions of oil palm fruits were significantly upgraded after being treated by UHP [12]. However, the influence of UHP treatment on the pancreatic lipase and α-glucosidase inhibitory activities of the different phenolic fractions from oil palm fruits is still unclear.

Therefore, the pancreatic lipase and α-glucosidase inhibitory activities of the different phenolic fractions (F, *E*, and IB) from nontreated and UHP-treated oil palm fruits were comparatively evaluated, and then the interactions between the two main phenolic compounds (detected in all phenolic fractions) on inhibiting lipase and α-glucosidase activities were further investigated. Finally, molecular docking analyses were used to delineate the underlying inhibition mechanisms of those two predominant phenolic compounds.

2. Materials and Methods

2.1. Chemicals and Reagents. Pancreatic lipase (from porcine pancreas, 163 U/mg, EC: 3.1.1.3), α-glucosidase (from *Saccharomyces cerevisiae*, Type I, ≥10 U/mg protein), *p*-nitrophenyl laurate (PNP), *p*-nitrophenyl-α-D-glucopyranoside (*p*-NPG), acarbose, and orlistat were purchased from Sigma (Sigma-Aldrich, Shanghai, China). The purity of authentic standards of phenolic compounds (catechin and caffeic acid) were ≥98% and obtained from Chengdu Must Bio-Technology Co., Ltd. (Chengdu, China). Other reagents used were of analytical grade.

2.2. Preparation and UHPLC-MS/MS Analysis of Samples. Oil palm fruits were obtained in 2017, from a local market (Haikou City, Hainan Province, P.R. China) and kept at low temperature (4°C–6°C) throughout transportation and stored at −20°C after being received. The methods used for UHP pretreatment of oil palm fruits and the extraction of F, *E*, and IB phenolic fractions were performed in the same way as in our previous report [12]. Mesocarps were vacuum-packed before UHP treatment and then processed with HHP-600 (Baotou Kefa High Pressure Technology Limited Company, Baotou, China) for 10 min at 500 Mpa. Then, all samples were lyophilized and smashed into powder with a high-speed grinder (Lingdan LD-T300, Shanghai, China) for extraction. The phytochemical compositions of *F*, *E*,, and IB phenolic fractions were analyzed by UHPLC-MS/MS, which were similar with the results reported in our previous work [12]; catechin and caffeic acid were the main phenolic compounds detected in all phenolic fractions of non- and UHP-treated fruits.

2.3. Inhibition of Pancreatic Lipase Activity. The assay of pancreatic lipase inhibition was performed according to the method reported earlier [14]. Briefly, pancreatic lipase was dissolved into 150 mg/mL with distilled water, and the supernatant was obtained by centrifuged with 10,000 g for 5 minutes at 4°C. The 1% PNP and 1% Triton X-100 were added in sodium acetate solution (5 mM pH 5.0) to prepare the substrate solution. The absorbance value of each sample was obtained at 400 nm by a SpectraMax M5 microplate reader (Molecular device, USA). All the experiments were conducted three times.

2.4. Inhibition of α-Glucosidase Activity. The inhibition of α-glucosidase activity was conducted as per a previously described method [15]. In this assay, *p*-NPG served as substrate, which was dissolved into 2.5 mM with PBS (PH = 6.8). α-Glucosidase solution also prepared with PBS, by dissolving 1 mg α-glucosidase with 0.5 mL PBS and then diluting ten times when needed. The absorbance value of each reaction mixture was measured at 405 nm by a SpectraMax M5 microplate reader. All the experiments were conducted three times.

2.5. Interaction of Catechin and Caffeic Acid on Inhibition of Pancreatic Lipase and α-Glucosidase. According to the phytochemical analysis [12], catechin and caffeic acid were the predominant phenolic compounds detected in all samples. Therefore, the interaction of catechin and caffeic acid on inhibition of pancreatic lipase and α-glucosidase was determined by CalcuSyn software (Biosoft, Ferguson, MO, USA) as in the previous report [16]. For facilitating calculation and analysis of phenolic interaction types, the inhibitory effects of combined catechin and caffeic acid on pancreatic lipase and α-glucosidase were evaluated by three different ratios, 2 : 1, 1 : 2, and 1 : 1, respectively. The interaction types were evaluated based on CI (combination index; $CI \leq 0.90$: synergistic effect; $0.90 < CI < 1.10$: additive effect; and $CI \geq 1.10$: antagonistic effect).

2.6. Molecular Docking. Molecular docking of pancreatic lipase and α-glucosidase with phenolic standards (catechin and caffeic acid) was conducted by SYBYL-X 2.1.1 (Tripos, Inc., St. Louis, MO, USA) according to the previous methods [14, 15]. The 3D configurations of the catechin and caffeic acid compounds were obtained from PubChem (http://www.ncbi.nlm.nih.gov/pccompound), and the 3D structure of lipase was downloaded from RCSB PDB (http://www.rcsb.org/pdb/home.do). Since the 3D structure of α-glucosidase of *S. cerevisiae* is unavailable, isomaltase (PDB code: 3A4A) from the same organism, which shares high similarity with α-glucosidase, were used for homology modeling in the molecular docking analysis in this study.

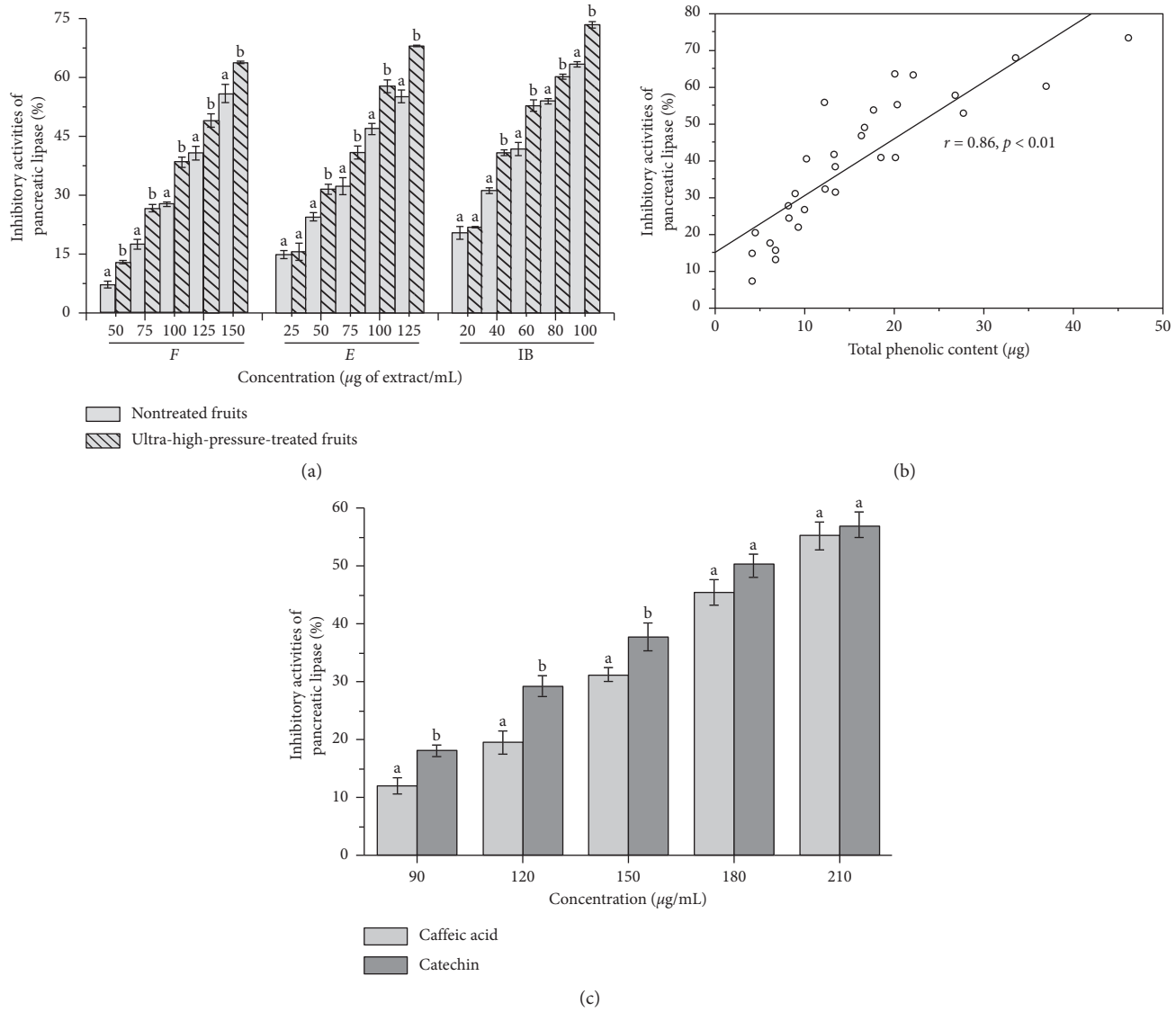


Figure 1: (a) The lipase inhibitory effects of free, esterified and insoluble-bound phenolic fractions from nontreated and ultra-high-pressure-treated oil palm fruits. (b) The correlation analysis of TPC and pancreatic lipase inhibitory effects. (c) Two main phenolic compound standards (catechin and caffeic acid). "*F*": free phenolic fraction; "*E*": esterified phenolic fraction; "IB": insoluble-bound phenolic fraction.

2.7. Statistical Analysis. Each experiment was conducted three times, and results were expressed as mean ($n = 3$) ± standard deviation (SD). The significance analysis ($p < 0.05$) of all data was conducted by one-way ANOVA and Tukey's procedure by using Origin 8.5 software (OriginLab, Northampton, MA, USA). Pearson's correlation coefficient was performed by SPSS 20.0 (SPSS, Inc., Chicago, IL, USA).

3. Results and Discussion

3.1. Lipase Inhibition of Extracts from Nontreated and UHP-Treated Oil Palm Fruits. The results of lipase inhibitory effects of the extracts from nontreated and UHP-treated oil palm fruits are presented in Figure 1(a). All of phenolic fractions, regardless of UHP treatment, showed good inhibitory activities towards the pancreatic lipase and presented in a dose-dependent manner at the tested concentrations. Among the three fractions of nontreated oil palm fruits, the insoluble-bound phenolic fraction showed the strongest inhibition, with the IC50 value of 78.01 ± 1.04 μg/mL. The following is the esterified phenolic fraction, whose IC50 value was 112.24 ± 1.63 μg/mL, and the free phenolic fraction possessed the weakest inhibition, i.e., IC50 = 141.01 ± 2.43 μg/mL. As for UHP-treated oil palm fruits, the inhibitory effects coincided with the order shown in nontreated oil palm fruits, insoluble-bound phenolic fraction > esterified phenolic fraction > free phenolic fraction, and their IC50 value was 72.50 ± 1.36 μg/mL, 95.68 ± 2.37 μg/mL, and 127.57 ± 2.17 μg/mL, respectively. The insoluble-bound phenolic fraction showed the highest inhibitory rate, regardless of UHP pretreatment, which may

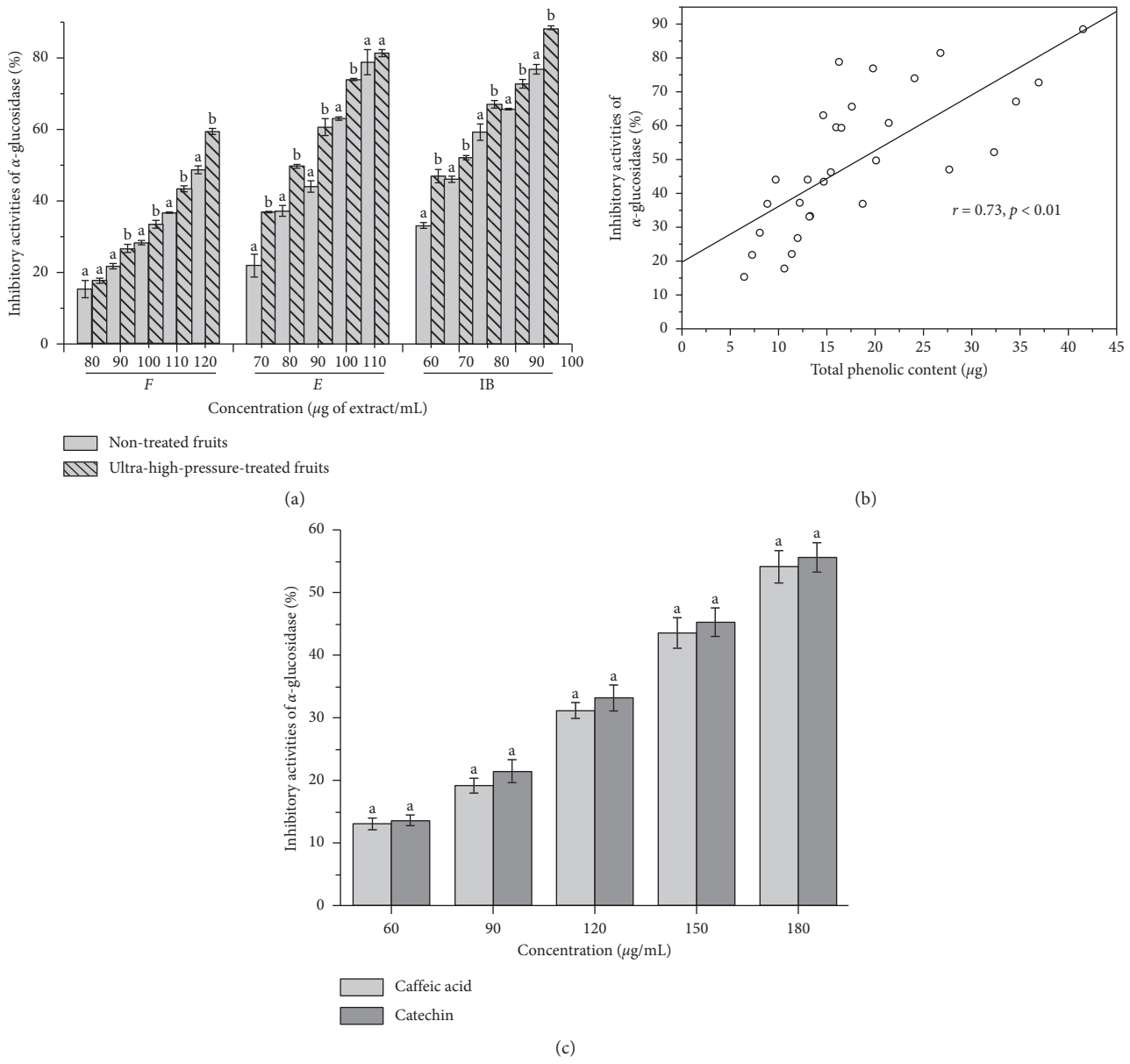


Figure 2: (a) The α-glucosidase inhibitory effects of free, esterified, and insoluble-bound phenolic fractions from nontreated and ultra-high-pressure-treated oil palm fruits. (b) The correlation analysis of TPC and α-glucosidase inhibitory effects. (c) Two main phenolic compound standards (catechin and caffeic acid). "*F*": free phenolic fraction; "*E*": esterified phenolic fraction; "IB": insoluble-bound phenolic fraction.

be because the composition of this phenolic fraction was abundant and the content was comparatively higher than other two parts according to our previous report [12]. A previous study also reported that the insoluble-bound phenolic extract of Sophia seeds had the best inhibitory effect against pancreatic lipase [17]. However, some other studies found that the free phenolic fraction of chia meal or Chinese sumac fruits had the strongest inhibition towards lipase [14, 18]. Those results indicated that the inhibitory effect of lipase may be profoundly affected by the differences of phenolic composition or raw material. Compared with nontreated oil palm fruits, the UHP pretreatment significantly enhanced the inhibitory effect ($p < 0.05$). After UHP treatment, the IC_{50} values of free, esterified, and insoluble-bound phenolic fractions were declined by about 7.06%, 14.75%, and 9.53%, respectively. Cai et al. [19] reported fermentation could also be used as an efficient method to enhance the inhibition of oat towards the pancreatic lipase.

Many previous studies have indicated that there was a close correlation between enzyme inhibition and total phenolic content (TPC) [19,20]. In this work, a positive relationship between lipase inhibitory rates and TPC was also observed ($r = 0.86, p < 0.01$, Figure 1(b)), which suggested phenolic compounds may also respond to the lipase inhibition of the oil palm fruits. According to our previous work, catechin and caffeic acid were the main phenolics presented in all six phenolic fractions of nontreated and UHP-treated oil palm fruits [12]. As seen in Figure 1(c),

these two phenolic standards also exhibited good inhibitory effects against lipase in a dose-dependent manner. The IC_{50} values of catechin and caffeic acid were $189.02 \pm 5.56\,\mu g/mL$ and $197.26 \pm 4.40\,\mu g/mL$ respectively, both of which were significant higher than that of orlistat (positive control, $IC_{50} = 46.22 \pm 0.84\,\mu g/mL$) ($p < 0.05$) and had no significant difference with each other ($p > 0.05$). While, in Tan et al. [21] report, the IC_{50} values of caffeic and catechin acid were much higher than our values obtained in this study, with the IC_{50} values of 0.94 ± 0.09 mg/mL and 0.53 ± 0.05 mg/mL, respectively. In another report, the IC_{50} value of caffeic acid was $32.60\,\mu g/mL$ [5]. These discrepancies may be caused by the differences of assay methods and enzyme batch used.

3.2. α-Glucosidase Inhibition of Extracts from Nontreated and UHP-Treated Oil Palm Fruits. The α-glucosidase inhibitory results of different phenolic fraction from nontreated and UHP-treated oil palm fruits are presented in Figure 2(a). It is apparent that all the phenolic fractions displayed inhibition to α-glucosidase in a dose-dependent manner. In terms of nontreated oil palm fruits, the inhibitory tendency of the free, esterified, and insoluble-bound phenolic fraction towards α-glucosidase was similar to that towards pancreatic lipase; the insoluble-bound phenolic fraction possessed the strongest inhibition, followed by esterified fraction, while the free fraction had the weakest inhibitory effect ($p < 0.05$). The IC_{50} values of those three fractions were $76.42 \pm 0.94\,\mu g/mL$, $93.42 \pm 1.53\,\mu g/mL$, and $122.82 \pm 2.01\,\mu g/mL$, respectively. The UHP pretreatment significantly enhanced the α-glucosidase inhibitory effects of the three different phenolic fractions and the insoluble-bound phenolic fraction also showed the strongest inhibition ($p < 0.05$). The IC50 values of insoluble-bound, esterified, and free phenolic fractions were $64.51 \pm 2.41\,\mu g/mL$, $82.43 \pm 0.65\,\mu g/mL$, and $114.10 \pm 0.96\,\mu g/mL$, respectively, which were enhanced by about 15.58%, 11.76%, and 7.10%, respectively, by comparison with the corresponding fraction without UHP. A previous study showed that esterified phenolic of pomegranate seeds possessed the strongest α-glucosidase inhibitory effect [22]. Another study reported that the inhibitory effect of chia meal-free phenolic against α-glucosidase was the strongest [18]. Therefore, the inhibitory effects of α-glucosidase largely depend on phenolic composition and raw materials. In this work, UHP pretreatment significantly enhanced the α-glucosidase inhibitory capacity. Feng et al. [23] found that solid-state fermentation also greatly increased the α-glucosidase inhibition of tartary buckwheat.

There is also a clear correlation between α-glucosidase inhibitory rates and TPC in the present work ($r = 0.73$, $p < 0.01$), suggesting phenolic compounds may take a responsibility to the α-glucosidase inhibition of the oil palm fruits (Figure 2(b)). Catechin and caffeic acid, as the primary phenolics in all sample, showed good α-glucosidase inhibitory activities with IC_{50} values of $163.07 \pm 3.93\,\mu g/mL$ and $165.29 \pm 5.47\,\mu g/mL$, respectively (Figure 2(c)). According to the IC_{50} values of these two phenolics, they showed a similar efficiency on inhibiting the α-glucosidase and also had no significant difference with each other ($p > 0.05$). However, their inhibitory effects were dramatically weaker than that of acarbose ($IC_{50} = 5.83 \pm 0.65\,\mu g/mL$) ($p < 0.05$).

3.3. Interactions between Catechin and Caffeic Acid on Inhibition of Pancreatic Lipase and α-Glucosidase. As shown in the above results of pancreatic lipase or α-glucosidase inhibition, the inhibitory effect of single phenolic was significantly weaker than that of any of the phenolic fractions. According to our previous work, several phenolics have been detected in oil palm fruits, and each phenolic fraction included more than one compound [12]. So, we speculated that the interaction between different phenolics may be taken when evaluating the enzyme inhibitory effects of different phenolic fractions. Since catechin and caffeic acid were two main phenolics detected in all phenolic fractions of nontreated and UHP-treated oil palm fruits [12], the interaction between these two phenolic compounds was evaluated by CalcuSyn software (Biosoft, Ferguson, MO, USA). Three different combination ratios, 1 : 2, 2 : 1, and 1 : 1, were used to explore the interaction types, and the best combination ratio was found. The interacted inhibitory effects of catechin and caffeic acid on pancreatic lipase are presented in Table 1 and on α-glucosidase are summarized in Table 2. According to the results of pancreatic lipase inhibition (Table 1), when catechin and caffeic acid are combined at a ratio of 1 : 2, the CI value gradually decreases with the increasing concentration, and interaction type between catechin and caffeic acid varies from antagonistic (low concentration) to additive effect (high concentration). When the combination ratio of catechin and caffeic acid was 2 : 1, they showed the same trend as observed at the ratio of 1 : 2. When catechin and caffeic acid was combined at a ratio of 1 : 1, the synergistic inhibitory effect against pancreatic lipase was observed at relative high concentrations (185 and $210\,\mu g/mL$).

Results of interactions between catechin and caffeic acid on inhibiting αα-glucosidase at different combination ratios, which are presented in Table 2, exhibit similar phenomena in the findings observed in lipase inhibition assay. As shown in 2, when catechin and caffeic acid were combined at the ratio of 1 : 2, the CI value decreased with the increasing concentration. At the highest concentration, the interaction type between those two phenolic compounds transformed from antagonistic effect into addictive effect. Similar behaviors found at ratio of 1 : 2 was also observed when the combination ratio between catechin and caffeic acid was set at 2 : 1. When catechin and caffeic acid were combined at a ratio of 1 : 1, the interaction type varied from additive effect to synergistic effect on inhibiting α-glucosidase with increasing concentration. Many previous literatures have reported that the combined samples work better than each sample working alone [24, 25]. Zhang et al. [16] reported that combining myricetin-3-*O*-rhamnoside and quercetin-3-*O*-rhamnoside dramatically increased the inhibitory effect to lipase. The present work also suggested that interaction between different phenolic compounds on inhibiting pancreatic lipase and α-glucosidase may happen, and the

Table 1: The interactions between catechin and caffeic acid on inhibition of pancreatic lipase.

Combination doses	Catechin	Caffeic acid	Inhibition ratio	CI[a]	Fa[b]	CI of Fa	Catechin	Caffeic acid
	Combination test (μg/mL)		(%)		(%)		Calculation at corresponding Fa (μg/mL)	
	45.0	90.0	13.70 ± 0.23	1.27	25.0	1.16	62.88	125.76
	60.0	120.0	21.00 ± 1.63	1.22	50.0	1.01	97.54	195.09
1 : 2	75.0	135.0	32.30 ± 1.22	1.14	75.0	0.93	151.31	302.63
	90.0	185.0	46.17 ± 1.21	1.08	90.0	0.87	234.72	469.45
	105.0	210.0	55.76 ± 2.14	0.99				
	90.0	45.0	15.14 ± 1.36	1.24	25.0	1.17	121.38	60.69
	120.0	60.0	22.45 ± 1.99	1.19	50.0	0.98	190.72	95.36
2 : 1	135.0	75.0	33.74 ± 2.10	1.10	75.0	0.87	299.68	149.84
	185.0	90.0	47.61 ± 1.82	1.05	90.0	0.83	470.89	235.44
	210.0	105.0	57.21 ± 2.53	0.92				
	90.0	90.0	41.64 ± 2.31	1.06	25.0	1.15	45.11	45.11
	120.0	120.0	54.10 ± 1.44	0.97	50.0	0.99	113.64	113.64
1 : 1	135.0	135.0	57.40 ± 1.92	0.95	75.0	0.84	286.28	286.28
	185.0	185.0	64.28 ± 2.13	0.90	90.0	0.78	521.24	521.24
	210.0	210.0	66.43 ± 2.37	0.88				

[a]CI, combination index. CI ≤ 0.90, 0.90 < CI < 1.10, or CI ≥ 1.10 represent synergistic, additive, and antagonistic effects, respectively. [b]Fa, fraction affected means the dose of combination of catechin and caffeic acid effect on inhibition rate. CI25, CI50, CI75, and CI90 represent the corresponding CI value when Fa is 25%, 50%, 75%, and 90%.

Table 2: The interactions between catechin and caffeic acid on inhibition of α-glucosidase

Combination doses	Catechin	Caffeic acid	Inhibition ratio	CI[a]	Fa[b]	CI of Fa	Catechin	Caffeic acid
	Combination test (μg/mL)		(%)		(%)		Calculation at corresponding Fa (μg/mL)	
	30.0	60.0	12.42 ± 0.39	1.30	25.0	1.21	48.47	96.95
	45.0	90.0	20.41 ± 1.03	1.24	50.0	1.09	84.48	168.96
	60.0	120.0	32.24 ± 1.76	1.16	75.0	0.95	147.24	294.48
1 : 2	75.0	135.0	44.50 ± 1.43	1.12	90.0	0.89	256.62	513.23
	90.0	180.0	54.91 ± 1.82	1.07				
	60.0	30.0	15.05 ± 0.62	1.28	25.0	1.20	89.12	44.56
	90.0	45.0	23.04 ± 0.88	1.22	50.0	1.02	160.43	80.22
	120.0	60.0	34.87 ± 1.71	1.13	75.0	0.94	288.81	144.41
	150.0	75.0	47.13 ± 1.46	1.09	90.0	0.87	519.92	259.96
2 : 1	180.0	90.0	57.54 ± 2.03	1.04				
	60.0	60.0	34.68 ± 1.08	1.16	25.0	1.23	45.02	45.02
	90.0	90.0	43.83 ± 1.22	1.10	50.0	1.02	98.72	98.72
	120.0	120.0	56.73 ± 2.36	0.99	75.0	0.85	215.59	215.59
1 : 1	150.0	150.0	65.51 ± 2.14	0.93	90.0	0.76	470.83	470.83
	180.0	180.0	69.98 ± 1.75	0.90				

[a]CI, combination index. CI ≤ 0.90, 0.90 < CI < 1.10, or CI ≥ 1.10 represent synergistic, additive, and antagonistic effects, respectively. [b]Fa, fraction affected, means the dose of combination of catechin and caffeic acid effect on inhibition rate. CI_{25}, CI_{50}, CI_{75}, and CI_{90} represent the corresponding CI value when Fa is 25%, 50%, 75%, and 90%.

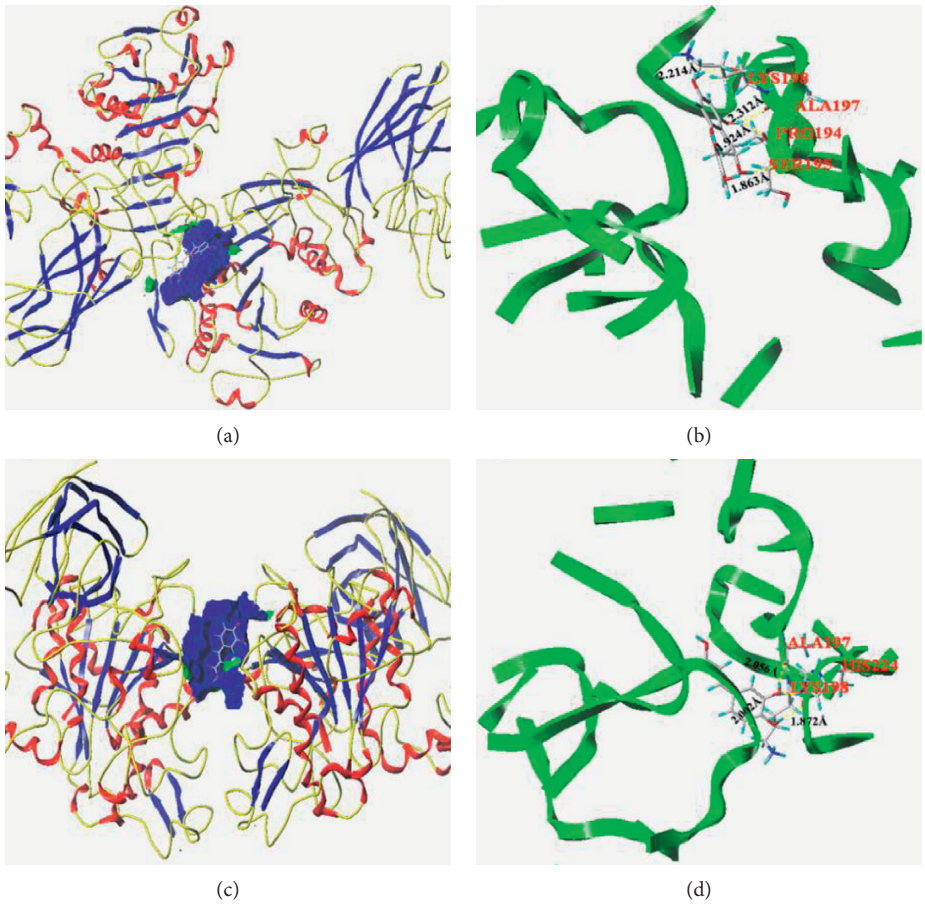
interaction type varies with the variation of the combination concentrations and ratios.

3.4. Molecular Docking Results of Pancreatic Lipase and α-Glucosidase. Computational modeling soft SYBYL-X 2.1.1 (Tripos, Inc., St. Louis, MO, USA) was used to analyze the enzyme inhibitory mechanism of catechin and caffeic acid. The interaction was evaluated by some relevant parameters, for example, C-Score and T-Score. The value of C-Score is used to evaluate if the docking result is credible, which should be no less than four, and the T-Score reveals the ability of binding affinity, which is an important index to value the docking results [14].

The docking results of pancreatic lipase are summarized in Table 3 and Figure 3(a). The C-Scores of two main phenolics (catechin and caffeic acid) were four, suggesting the docking results were effective and credible (Table 3). The T-Scores in Table 3 indicated that catechin may have a better binding affinity to lipase than caffeic acid. As shown in the Figures 3(a) and 3(b), catechin bound with four amino acid residues (Ala197, Pro194, Ser195, and Lys198) in the pancreatic lipase active site. Ala197, Pro194, and Ser195 served as an H-bond receptor, the hydrogen bond distances of

Table 3: The score results of two phenolic compounds docking with pancreatic lipase and α-glucosidase, respectively.

	C-Score	T-Score	PMF-Score	CHEM-Score	G-Score	D-Score
Pancreatic lipase						
Catechin	4	4.4351	27.2574	−15.5057	−131.1809	−85.8563
Caffeic acid	4	3.4707	22.5169	−17.6857	−113.1308	−67.2611
α-Glucosidase						
Catechin	5	4.8871	19.9048	−14.4315	−87.8383	−106.1603
Caffeic acid	4	4.0400	−13.7135	−13.0411	−51.2488	−74.2849



(a) (b) (c) (d)

Figure 3: The molecule docking results of catechin and caffeic acid with pancreatic lipase. (a) Catechin binds with the active site of pancreatic lipase. (b) Catechin interacts with amino acid residues. (c) Caffeic acid binds with the active site of pancreatic lipase. (d) Caffeic acid interacts with amino acid residues. Two phenolic standards appeared in ball and stick models, amino acid residues are represented by green capped stick models, and short dotted yellow line stands for hydrogen bonds.

which were 2.312 Å, 1.924 Å, and 1.863 Å, respectively. Lys198 was an H-bond donor, and the hydrogen bond length was 2.124 Å. The average hydrogen bond distance was 2.056 Å. In Figures 3(c) and 3(d), caffeic acid bound with the pancreatic lipase active site by interacting with three amino acid residues, namely, Ala197, His224, and Lys198. Ala197 and His224 served as an H-bond receptor, and Lys198 acted as an H-bond donor. The hydrogen bond distance between caffeic acid and these amino acid residues was 2.065 Å, 1.872 Å, and 2.022 Å, respectively. The average hydrogen bond distance was 1.986 Å. According to those results, both catechin and caffeic acid interacted with amino acid residues Lys198 and Ala197, suggesting these two amino acids may play a key role in the pancreatic lipase activity. Zhang et al. [15] also reported that cyandin-3-O-glucoside may exert its lipase inhibition through binding with Lys198 and Ala197. Another study reported that myricitrin and quercitrin interacted with Asp 80 and Gly 77 to inhibit the activity of pancreatic lipase [14]. According to those previous results and the current findings, they clearly indicated that different phenolic compounds may interact with different amino acids to exert their lipase inhibitory effects. Although T-scores indicated that catechin may have a better binding affinity to lipase than caffeic acid, the number and distance of hydrogen bonds are also very important for phenolics inhibiting the lipase activity. The more and/or the shorter

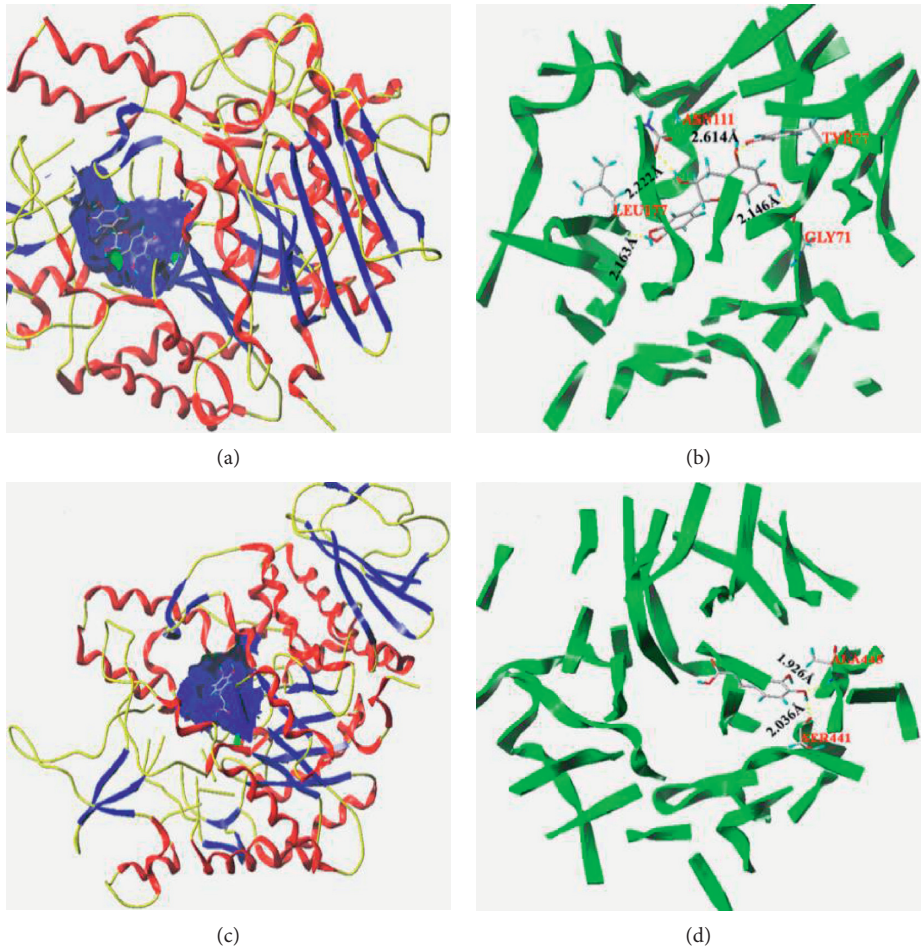


Figure 4: The molecule docking results of catechin and caffeic acid with α-glycosidase. (a) Catechin binds with the active site of α-glucosidase. (b) Catechin interacts with amino acid residues. (c) Caffeic acid binds with the active site of α-glucosidase. (d) Caffeic acid interacts with amino acid residues. Two phenolic standards appeared in ball and stick models, amino acid residues are represented by green capped stick models, and short dotted yellow line stands for hydrogen bonds.

hydrogen bonds represent a tighter binding and thereby a stronger inhibition [26]. In the present work, the average hydrogen bond distance of catechin was longer than that of caffeic acid, while the hydrogen bond number of catechin was one more than that of caffeic acid. The one more hydrogen bond of catechin may counteract the disadvantage of slight longer hydrogen bond distance of catechin on inhibiting pancreatic lipase, which could explain why catechin and caffeic acid had a similar efficiency on inhibiting lipase.

For molecular docking of α-glucosidase, the C-Score results of two phenolics were also no less than four (Table 3). The catechin also had a higher T-score than caffeic acid, indicating that catechin may also have a better binding affinity to α-glucosidase than caffeic acid (Table 3). In Figures 4(a) and 4(b), four hydrogen bonds were formed between catechin and four amino acids (Leu177, Asn111, Tyr77, and Gly71) in the α-glucosidase active pocket. Among the three H-bond receptors (Leu177, Asn111, and Gly71), the Asn111 possessed the longest hydrogen bond with a distance of 2. 222 Å, and the hydrogen bond distances of Leu177 and Gly71 were 2.163 Å and 2.146 Å, respectively. The Tyr77 acted as an H-bond donor, and the hydrogen bond distance was 2.614 Å. The average distance of these four hydrogen bonds was 2.286 Å. Two amino acid residues were observed between caffeic acid and α-glucosidase active pocket at Figures 4(c) and 4(d), namely, Ala445 and Ser441. Ala445, as an H-bond donor, generated an H-bond with caffeic acid with a distance of 1.926 Å, while Ser441 served as an H-bond receptor and the hydrogen bond length was 2.036 Å. The average hydrogen bond distance was 1.981 Å. The average hydrogen bond distance of caffeic acid was much shorter than that of catechin, while the hydrogen bond number of catechin was two more than that of caffeic acid. As mentioned above in the docking analysis of pancreatic lipase, the disadvantage of longer hydrogen bond of catechin on inhibiting α-glucosidase may be remedied by more hydrogen bond number when compared with that of caffeic acid. Therefore, catechin and caffeic acid had a similar efficiency on inhibiting α-glucosidase. A previous study found that both rutin and isorhamnetin-3-*O*-rutinoside formed H-bonds with the amino acid residues Ile 440, Arg 213, and Arg 315 of α-glucosidase [15]. Another study found that the binding sites of apigenin were Ser311 and Gly309

[26]. Gallocatechin gallate was reported to form hydrogen bonds with Arg 315 and Phe 303 to effectively inhibit the catalytic activity of α-glucosidase [27]. Those different results also obviously suggested that the structures of phenolic compounds may profoundly affect their binding sites on α-glucosidase.

4. Conclusions

The results of the present study showed that the free, esterified, and insoluble-bound phenolic fractions from nontreated and UHP-treated oil palm fruits demonstrated good inhibitory effects towards pancreatic lipase and α-glucosidase. Moreover, the inhibitory effects of these three different phenolic fractions on lipase and α-glucosidase were significantly upgraded after being pretreated with UHP ($p < 0.05$). The insoluble-bound phenolic fraction, regardless of UHP treatment, presented the strongest enzyme inhibitory capacities, both in lipase and α-glucosidase. Catechin and caffeic acid, as the main phenolic compounds detected in all samples, also showed a dose-dependent inhibition toward the two enzymes and exhibited a synergistic effect on inhibiting pancreatic lipase and α-glucosidase at a high combination concentration with the ratio of 1 : 1. Moreover, catechin and caffeic acid showed similar efficiencies on inhibiting the two digestive enzymes, which were consistent with the findings observed by molecular docking analysis. This study suggested that different phenolic extracts of oil palm fruits, especially the UHP-treated fruits, could be potentially developed as nutraceuticals or functional foods for controlling or alleviating diseases related to over intake of glucose and/or lipid, such as diabetes, hyperlipidemia, cardiovascular diseases, and obesity.

Data Availability

All data included in this study are available upon request by contacting the corresponding author.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Qingfeng Zhou and Jiexin Zhou contributed equally to this work.

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References

[1] S. Meng, J. Cao, Q. Feng, J. Peng, and Y. Hu, "Roles of chlorogenic acid on regulating glucose and lipids metabolism: a review," *Evidence-Based Complementary And Alternative Medicine*, vol. 2013, Article ID 801457, 11 pages, 2013.

[2] M. Kohno, H. Sugano, Y. Shigihara, Y. Shiraishi, and T. Motoyama, "Improvement of glucose and lipid metabolism via mung bean protein consumption: clinical trials of GLUCODIA™ isolated mung bean protein in the USA and Canada," *Journal of Nutritional Science*, vol. 7, no. e2, pp. 1–11, 2018.

[3] V. G. DeMarco, A. R. Aroor, and J. R. Sowers, "The pathophysiology of hypertension in patients with obesity," *Nature Reviews Endocrinology*, vol. 10, no. 6, pp. 364–376, 2014.

[4] W. Kooti, M. Farokhipour, Z. Asadzadeh, D. Ashtary-Larky, and M. Asadi-Samani, "The role of medicinal plants in the treatment of diabetes: a systematic review," *Electronic Physician*, vol. 8, no. 1, pp. 1832–1842, 2016.

[5] Y. Bustanji, A. Issa, M. Mohammad et al., "Inhibition of hormone sensitive lipase and pancreatic lipase by *Rosmarinus officinalis* extract and selected phenolic constituents," *Journal of Medicinal Plants Research*, vol. 4, no. 21, pp. 2235–2242, 2010.

[6] P. Taslimi, C. Caglayan, and I. Gulcin, "The impact of some natural phenolic compounds on carbonic anhydrase, acetylcholinesterase, butyrylcholinesterase, and α-glycosidase enzymes: an antidiabetic, anticholinergic, and antiepileptic study," *Journal of Biochemical and Molecular Toxicology*, vol. 31, no. 12, 2017.

[7] A. L. La Garza, F. I. Milagro, N. Boque, J. Campion, and J. A. Martinez, "Natural inhibitors of pancreatic lipase as new players in obesity treatment," *Planta Medica*, vol. 77, no. 8, pp. 773–785, 2011.

[8] C. Kecyk and L. Zubach-Cassano, "Natural health products and diabetes: a practical review," *Canadian Journal of Diabetes*, vol. 41, no. 6, pp. 642–647, 2017.

[9] Q. You, F. Chen, X. Wang, Y. Jiang, and S. Lin, "Anti-diabetic activities of phenolic compounds in muscadine against alpha-glucosidase and pancreatic lipase," *Lwt-Food Science and Technology*, vol. 46, no. 1, pp. 164–168, 2012.

[10] P. Ye, R. Gan, H. Li et al., "Absorption, metabolism, and bioactivity of vitexin: recent advances in understanding the efficacy of an important nutraceutical," *Critical Reviews in Food Science and Nutrition*, pp. 1–16, 2020, In press.

[11] J. C. Kurnia, S. V. Jangam, S. Akhtar, A. P. Sasmito, and A. S. Mujumdar, "Advances in biofuel production from oil palm and palm oil processing wastes: a review," *Biofuel Research Journal*, vol. 3, no. 1, pp. 332–346, 2016.

[12] J. Zhou, Y. Ma, Y. Jia, M. Pang, G. Cheng, and S. Cai, "Phenolic profiles, antioxidant activities and cytoprotective effects of different phenolic fractions from oil palm (*Elaeis guineensis* Jacq.) fruits treated by ultra-high pressure," *Food Chemistry*, vol. 288, pp. 68–77, 2019.

[13] H.-W. Huang, S.-J. Wu, J.-K. Lu, Y.-T. Shyu, and C.-Y. Wang, "Current status and future trends of high-pressure processing in food industry," *Food Control*, vol. 72, no. 72, pp. 1–8, 2017.

[14] C. Zhang, Y. Ma, F. Gao, Y. Zhao, S. Cai, and M. Pang, "The free, esterified, and insoluble-bound phenolic profiles of rhus chinensis mill. fruits and their pancreatic lipase inhibitory activities with molecular docking analysis," *Journal of Functional Foods*, vol. 40, pp. 729–735, 2018.

[15] X. Zhang, Y. Jia, Y. Ma, G. Cheng, and S. Cai, "Phenolic composition, antioxidant properties, and inhibition toward digestive enzymes with molecular docking analysis of different fractions from prinsepia utilis royle fruits," *Molecules*, vol. 23, no. 12, p. 3373, 2018.

[16] C. Zhang, Y. Ma, Y. Zhao, Y. Hong, S. Cai, and M. Pang, "Phenolic composition, antioxidant and pancreatic lipase inhibitory activities of Chinese sumac (Rhus chinensis Mill.)

fruits extracted by different solvents and interaction between myricetin-3-O -rhamnoside and quercetin-3-O -rhamnoside," *International Journal of Food Science & Technology*, vol. 53, no. 4, pp. 1045–1053, 2018.

[17] M. J. Rahman, P. Ambigaipalan, and F. Shahidi, "Biological activities of camelina and sophia seeds phenolics: inhibition of ldl oxidation, dna damage, and pancreatic lipase and α-glucosidase activities,," *Journal of Food Science*, vol. 83, no. 1-3, pp. 237–245, 2018.

[18] M. J. Rahman, A. C. De Camargo, and F. Shahidi, "Phenolic and polyphenolic profiles of chia seeds and their in vitro biological activities," *Journal of Functional Foods*, vol. 35, pp. 622–634, 2017.

[19] S. Cai, O. Wang, M. Wang et al., "In vitro inhibitory effect on pancreatic lipase activity of subfractions from ethanol extracts of fermented oats (avena sativa l.) and synergistic effect of three phenolic acids," *Journal of Agricultural and Food Chemistry*, vol. 60, no. 29, pp. 7245–7251, 2012.

[20] E. Apostolidis and C. M. Lee, "In vitro potential of ascophyllum nodosum phenolic antioxidant-mediated α-glucosidase and α-amylase inhibition," *Journal of Food Science*, vol. 75, no. 3, pp. H97–H102, 2010.

[21] Y. Tan, S. K. C. Chang, and Y. Zhang, "Comparison of α-amylase, α-glucosidase and lipase inhibitory activity of the phenolic substances in two black legumes of different genera," *Food Chemistry*, vol. 214, pp. 259–268, 2016.

[22] P. Ambigaipalan, A. C. De Camargo, and F. Shahidi, "Identification of phenolic antioxidants and bioactives of pomegranate seeds following juice extraction using HPLC-DAD-ESI-MS^n," *Food Chemistry*, vol. 221, pp. 1883–1894, 2017.

[23] L. Feng, Y. Xie, C. Peng, Y. Liu, and H. Wang, "A novel antidiabetic food produced via solid-state fermentation of tartary buckwheat by L. Plantarum TK9 and L. Paracasei TK1501," *Food Technology and Biotechnology*, vol. 56, no. 3, pp. 373–380, 2018.

[24] A.-S. Bustos, A. Håkansson, J. A. Linares-Pastén, J. M. Penarrieta, and L. Nilsson, "Interaction between phenolic compounds and lipase: the influence of solubility and presence of particles in the IC50 value," *Journal of Food Science*, vol. 83, no. 8, pp. 2071–2076, 2018.

[25] A. K. Singh, N. Kushwaha, and S. Chakraborty, "Synergistic interaction among begomoviruses leads to the suppression of host defense-related gene expression and breakdown of resistance in chilli," *Applied Microbiology and Biotechnology*, vol. 100, no. 9, pp. 4035–4049, 2016.

[26] L. Zeng, G. Zhang, S. Lin, and D. Gong, "Inhibitory mechanism of apigenin on α-glucosidase and synergy analysis of flavonoids," *Journal of Agricultural and Food Chemistry*, vol. 64, no. 37, pp. 6939–6949, 2016.

[27] X. Wu, H. Ding, H. Xing et al., "Exploring inhibitory mechanism of gallocatechin gallate on a -amylase and α-glucosidase relevant to postprandial hyperglycemia," *Journal of Functional Foods*, vol. 48, pp. 200–209, 2018.