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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

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ABSTRACT: The purpose of the present work is to study the pancreatic lipase inhibitory effects of different subfractions (*n*-hexane, ethyl acetate (EA), *n*-butanol, and water) from ethanol extracts of nonfermented and fungi-fermented oats and to delineate the interactions of three primary phenolic acids in the EA subfractions. The EA subfraction showed the highest inhibitory effect on pancreatic lipase activity at 1.5 mg/mL compared to the other subfractions, regardless of whether the oats were fermented. Meanwhile, both of the EA subfractions of two fungi-fermented oats demonstrated more effective inhibitory activity than that of nonfermented oats. A positive correlation between the total phenolics content and inhibitory activity was found. The inhibitory ability of the EA subfraction from nonfermented or fermented oats also displayed a dose-dependent effect. The standards of caffeic, ferulic, and *p*-coumaric acids, mainly included in EA subfractions of fermented oats, also displayed a dose-dependent inhibitory effect. A synergistic effect of each binary combination of *p*-coumaric, ferulic, and caffeic acids was observed, especially at 150.0 μg/mL. Those results indicate that fungi-fermented oats have a more effective inhibitory ability on pancreatic lipase and polyphenols may be the most effective component and could be potentially used for dietary therapy of obesity.

KEYWORDS: *inhibition of pancreatic lipase, synergistic effect, caffeic acid, ferulic acid, p-coumaric acid, oats*

INTRODUCTION

A recent study estimated that obesity among adults in the United Kingdom and United States has tripled and doubled over the past two decades, respectively.[1] More than 1.5 billion individuals are grouped as either overweight (body mass index (BMI) ≥ 25 kg/m^2) or obese (BMI ≥ 30 kg/m^2) around the world.[2] Previous studies also found that obesity is a key risk factor for morbidity from metabolic syndromes, such as diabetes, cardiovascular disease, and many other diseases.[3,4] Worldwide the prevalence of obesity continues to increase and has become a serious global health threat.

Substantial efforts to understand the physiological basis for and treatment of obesity have been made in the past decades. Hence, clearer understandings of mechanisms as well as several promising antiobesity drugs have been developed.[5] One of the most important strategies in the treatment of obesity is to reduce the intake of energy-dense fats. In humans, pancreatic lipase plays a key role in splitting triglycerides into bioavailable glycerol and fatty acids in the gastrointestinal tract.[6] Thus, suppression of pancreatic lipase by drugs, such as orlistat (which is one of two clinically approved drugs for treating obesity by the Food and Drug Administration (FDA)), has been employed to treat obesity.[7,8] Numerous studies also have been conducted by food scientists and nutritionists to search for more safe and effective inhibitors of pancreatic lipase from food materials in pursuit of this strategy.[7,9,10] Polyphenol-rich extracts, for example, have shown very good inhibitory effects on pancreatic lipase.[7,10] Gondoin et al.[9] also found that polyphenols in white and green tea can effectively inhibit pancreatic lipase in vitro, and the strictinin content in tea may be crucial for the inhibition of pancreatic lipase. Therefore, it is reasonable to speculate that food containing high contents of polyphenols may prove effective as inhibitors of pancreatic lipase activity.

Oats, widely recognized as a healthy food, are an important staple food for humans and contain numerous polyphenols. Caffeic and ferulic acids are typically the main phenolic acids in oats.[11] Vanillic, sinapic, *p*-coumaric, protocatechuic, and gallic acids are also found in oats.[12] Three major flavonoids have been identified in oats, namely, apigenin, luteolin, and tricin.[13] We previously reported that the total phenolics content (TPC) and total flavonoids in subfractions of oat (*Avena sativa* L.) solvent extracts increased dramatically after fermentation by various filamentous fungi, especially in ethyl acetate (EA) subfractions.[14] Therefore, one of the objectives of the present work is to evaluate whether subfractions of extracts of fermented oats have a better inhibitory effect on pancreatic lipase in comparison to their counterparts of nonfermented oats. Another aim of the present work is to determine which major phenolic acids in oats are involved in this bioactivity and to identify the efficiencies and interactions of those components.

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MATERIALS AND METHODS

Chemicals and Microorganisms. Caffeic acid, ferulic acid, *p*-coumaric acid, type 2 lipase of porcine pancreas (114 U/g), and *p*-nitrophenyl laurate (pNP laurate) were purchased from Sigma-Aldrich (Shanghai, China). The Folin−Ciocalteu reagent was purchased from Merck and Co., Inc. (Beijing China). Oats (genotype G4, harvested in 2010, Hebei Province, China), kept at 4 °C prior to use, were obtained from the Chinese Academy of Agricultural Sciences. Other reagents used were all of analytical grade (Beijing, China). Filamentous fungi (*Aspergillus oryzae* var. *effuses* and *A. oryzae*) used in this study were purchased from the Institute of Microbiology, Chinese Academy of Sciences (Beijing China), kept in lyophilized form, and activated in potato dextrose agar (PDA). The fungi were grown at 25 °C for 7 days to produce spores. Spore suspensions were made by suspending the spores from a PDA culture in 20 mL of 0.9% NaCl and kept at 4 °C for further use.

Preparation of Samples. Oats were treated and fermented according to our previous report.[14] In brief, oats were steeped in water at room temperature for 8 h and then smashed using an HK-06A high-speed grinder (Changsha, Hunan, China), sterilized at 121 °C for 15 min. The fermentation was conducted by adding a conidiospore suspension (10^6 spores/g of oats) into a 300 mL Erlenmeyer flask containing 50 g of smashed oats and then incubated at 25 °C for 3 days. During the cultivation period, the oats were stirred every 12 h. Similarly treated oats, except for the spore inoculation, were used as the control.

Different extracts were prepared by the protocol outlined previously.[14] Briefly, the nonfermented and fermented oat materials were ultrasonically extracted at 45 °C for 30 min with 80% ethanol. The slurries were centrifuged at 2862*g* for 15 min after being cooled to room temperature, and the supernatant was collected. Under the same conditions, the remainder was extracted once more, and the supernatants were combined. Then the solution was evaporated under reduced pressure at 45 °C to obtain the ethanol extracts. Thereafter, the ethanol extracts were dissolved in 80% methanol and sequentially extracted by *n*-hexane, EA, and water-saturated *n*-butanol by liquid−liquid partition. Four different subfractions were obtained after removal of the solvents, namely, *n*-hexane, EA, *n*-butanol, and water. The TPC in those extracts was determined by the Folin−Ciocalteu method.[15]

Pancreas Lipase Assay. The assay was performed according to previous reports[7,9] with some modification. In brief, lipase of porcine pancreas type 2 (Sigma product L3126, 114 U/g) was dissolved in ultrapure water at 5 mg/mL and then centrifuged at 10000*g* for 5 min, and the supernatant was used. The reaction buffer was 100 mM Tris buffer with pH 8.2. *p*-Nitrophenyl laurate (pNP laurate) was used as the reaction substrate. The substrate stock was 0.1% (w/v) pNP laurate dissolved in 5 mM sodium acetate (pH 5.0) containing 1% Triton X-100 and was heated in boiling water for 2 min to aid dissolution. After being mixed well, the stock was cooled to room temperature for further use. All test samples were dissolved in dimethyl sulfoxide (DMSO) and diluted to a proper concentration with 50% DMSO (DMSO:reaction buffer = 1:1). The control assay contained 200 μL of reaction buffer, 250 μL of substrate solution, 100 μL of 50% DMSO, and 150 μL of lipase. The 250 μL reaction substrate and 100 μL oat subfraction extracts were added to 200 μL of reaction buffer, and the enzyme was added to start the reaction. An inhibitor blank was made for each sample, which contained the reaction buffer, substrate, oat subfraction extract, and ultrapure water instead of the enzyme. The samples were incubated at 37 °C for 2 h and then centrifuged at 10000*g* for 1 min and their assays read at 400 nm using a multidetection microplate reader, SpetraMax M2[e]. All samples were assayed in triplicate. The results are expressed as inhibition (%) of lipase activity (OD = optical density): $(OD_{control} - OD_{sample})/OD_{control} \times 100$.

Interaction Assay of Standard Phenolic Acids on Lipase Activity. The interaction assay of these standard phenolic acids on lipase activity was performed according to previous reports[16−18] with some modifications. The OD value of the lipase reaction in the presence of phenolic acid A divided by that obtained in its absence was named V_a, which represents the remnant activity fraction of lipase after addition of A. The value for phenolic acid B (V_b) was defined in the same way as V_a. If the inhibitory effects on lipase of those two phenolic acids are independent, the final remnant activity fraction of lipase (V_c) is equal to V_aV_b when the reaction is sequentially treated by those two phenolic acids. The result of the OD value of the lipase reaction in the presence of both phenolic acids A and B divided by that obtained in their absence was named V_{ab}. The types of interaction were determined by the relationship between V_{ab} and V_a, V_b, or V_c as shown in Table 1 in detail. However, in the data analysis for determining the type of interaction, only the differences of $V_{ab} - V_c$ below −0.1 will be regarded as synergistic (SY), and the differences between −0.1 and +0.1 will be considered as additive (AD).

Table 1. Definitions of Interaction Type Determined by Different Variable Relationships between V_{ab} and V_a, V_b, or V_c

variable relationship between V_{ab}[a] and V_a,[b] V_b,[c] or V_c[d]	interaction type
$V_{ab} = V_c$	additive (AD)
$V_c < V_{ab}$ < the lower of V_a and V_b	subadditive
$V_{ab} < V_c$	synergistic (SY)
the lower of V_a and V_b < V_{ab} < the higher of V_a and V_b	interference
the higher of V_a and V_b < V_{ab}	antagonism
V_{ab} = the lower of V_a and V_b	indifference

[a]Remnant activity of lipase after addition of compounds A and B. [b]Remnant activity of lipase after addition of compound A. [c]Remnant activity of lipase after addition of compound B. [d]Result of V_aV_b.

Statistical Analysis. The data are expressed as the mean values ± standard deviation (SD) for each measurement. The data were also analyzed by one-way analysis of variance (one-way ANOVA). Tukey's procedure was used for the significance of difference ($p < 0.05$). The analysis was done with SPSS 13.0 (SPSS, Inc., Chicago, IL).

RESULTS

Inhibition of Lipase by Subfractions of Nonfermented and Fermented Oats. According to prework, four subfractions of nonfermented and fermented oats at 1.5 mg/mL were tested for their ability to inhibit pancreas lipase activity in vitro, and the results are summarized in Table 2. Among the four subfractions of nonfermented oats, the EA subfraction had the highest inhibitory rate on the lipase activity ($p < 0.05$), followed by the *n*-butanol and *n*-hexane subfractions. The water subfraction, however, had the lowest inhibition rate ($p < 0.05$). The inhibitory rate order of the four subfractions of each fermented oat is consistent with the order observed of the four subfractions of nonfermented oats, namely, EA > *n*-butanol > *n*-hexane > water. As shown in Table 2, the fermentation process significantly increased the inhibitory effect of EA subfractions ($p < 0.05$). In *A. oryzae* var. *effuses*-fermented oats, the inhibitory rate of the EA subfraction increased more than 2-fold when compared to that of nonfermented oats. The inhibitory rate of the EA subfraction of *A. oryzae*-fermented oats was also about 2 times higher than that of nonfermented oats, which was not statistically significantly different from that of *A. oryzae* var. *effuses*-fermented oats ($p > 0.05$). The fermentation process had no influence on the inhibitory rate of the *n*-hexane and water subfractions ($p > 0.05$). However, fermentation with these two fungi caused a slight but significant inhibition decrease of the *n*-butanol subfraction in comparison with the corresponding subfraction of nonfermented oats ($p < 0.05$).

The TPC of all samples (1.5 mg/mL) used in the inhibition tests was determined by the Folin−Ciocalteu method.

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Table 2. Pancreatic Lipase Inhibitory Effects of the Subfractions (1.5 mg/mL) from Ethanol Extracts of Nonfermented and Fermented Oats (*A. sativa* L.)[a]

	n-hexane	ethyl acetate	*n*-butanol	water
nonfermented oats	11.6 ± 1.8 a	34.7 ± 1.5 a	29.3 ± 0.4 a	8.7 ± 1.9 a
fermented with *A. oryzae* var. *effuses*	13.7 ± 2.3 a	70.6 ± 4.1 b	21.5 ± 1.8 b	10.8 ± 1.3 a
fermented with *A. oryzae*	13.0 ± 1.9 a	66.5 ± 3.3 b	22.2 ± 2.5 b	6.8 ± 1.9 a

[a]All values are expressed as inhibition (%) of lipase activity and as the mean ± SD (n = 3). Means in a column without online Roman letters in common differ significantly ($p < 0.05$).

Comparing the lipase inhibitory effect and the TPC of subfractions of nonfermented or fermented oats, it appears that the TPC may have a relationship with the inhibition of lipase activity. Taking all subfractions of nonfermented and fermented oats into account, there was a clear positive correlation between the TPC and inhibition rate (r = 0.921, $p < 0.01$) (Figure 1).

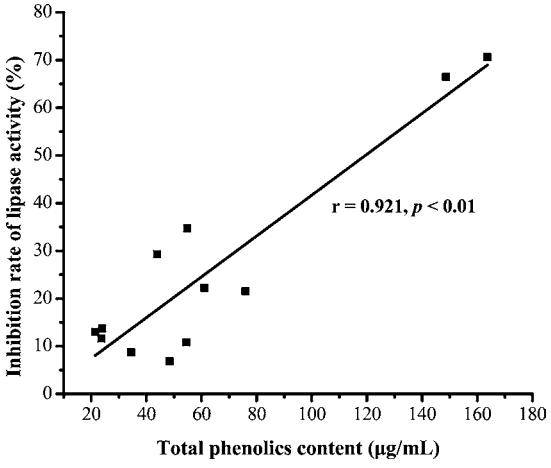

Figure 1. Correlation between the inhibitory rate of pancreatic lipase activity (%) and the TPC of the samples (μg/mL).

Inhibition of Lipase by EA Subfractions at Various Concentrations. Among the four subfractions, the EA subfractions had the highest inhibitory effect regardless of whether the oats were fermented. Inhibition of pancreas lipase by EA subfractions with various concentrations (from 0.25 to 2.0 mg/mL) was tested at the same conditions, and the results are presented in Figure 2. As shown in Figure 2, there was almost no inhibitory ability of the EA subfraction of nonfermented oats at 0.25 or 0.5 mg/mL. Moreover, the inhibition rate did not exceed 50% even when the concentration was at 2.0 mg/mL. However, the inhibition rate of the EA subfraction of nonfermented oats shows a dose-dependent relationship.

Just as the EA subfraction of nonfermented oats showed a dose-dependent inhibition, the EA subfractions of fermented oats also inhibits lipase activity in a dose-dependent manner. Both of the EA subfractions of the fungi-fermented oats inhibited about 80% of the lipase activity at 2.0 mg/mL. There was a statistically significant difference between the inhibition rates of EA subfractions from *A. oryzae* var. *effuses*-fermented oats and *A. oryzae*-fermented oats at low concentrations (below 1.5 mg/mL) ($p < 0.05$), and the EA subfraction of *A. oryzae* var. *effuses*-fermented oats was a more effective inhibitor than that of *A. oryzae*-fermented oats. However, the inhibition rate of the EA subfraction of *A. oryzae* var. *effuses*-fermented oats had no significant difference from that of *A. oryzae*-fermented oats at

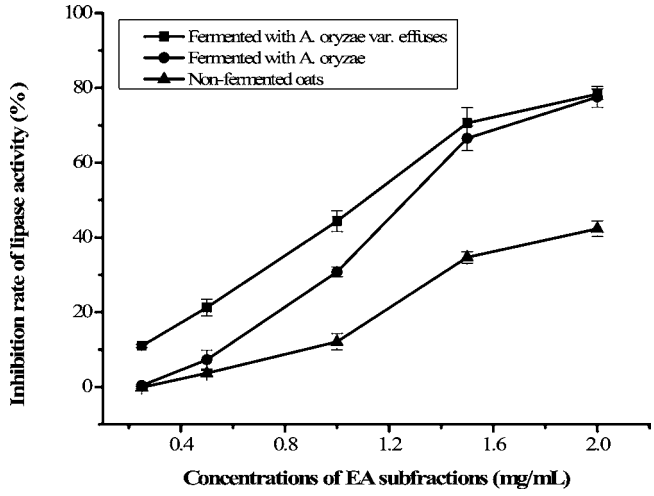

Figure 2. Inhibitory rate of pancreatic lipase activity at various concentrations of the EA subfractions from ethanol extracts of nonfermented and fermented oats (*A. sativa* L.). Values are expressed as the mean ± SD (n = 3).

1.5 or 2.0 mg/mL (Figure 2). The 50% maximal inhibitory concentrations (IC_{50}) of EA subfractions from those two fungi-fermented oats were1.17 ± 0.14 and 1.33 ± 0.22 mg/mL, respectively. Both of them were significantly lower than that of nonfermented oats (more than 2 mg/mL) ($p < 0.05$), especially the IC_{50} of the EA subfraction of *A. oryzae* var. *effuses*-fermented oats. The IC_{50} of orlistat was 29.3 ± 0.8 μg/mL (Figure 3).

Inhibition of Lipase by Three Phenolic Acids at Various Concentrations. Considering the high inhibitory effect on lipase activity of the EA subfractions which contained primarily *p*-coumaric, caffeic, and ferulic acids, the inhibitory

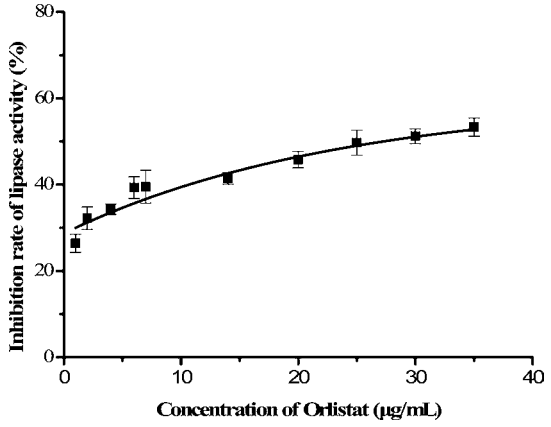

Figure 3. Inhibitory rate of pancreatic lipase activity at various concentrations of orlistat. Values are expressed as the mean ± SD (n = 3).

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ability of those three phenolic acids on lipase activity were tested using authentic standards at concentrations from 50.0 to 250.0 μg/mL, and the results are presented in Figure 4. As

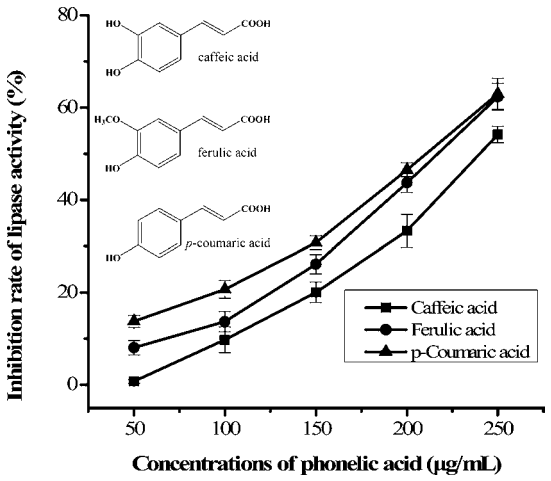

Figure 4. Inhibitory rate of pancreatic lipase activity at various concentrations of the three phenolic acid standards: (1) caffeic acid, (2) ferulic acid, (3) *p*-coumaric acid. Values are expressed as the mean ± SD (n = 3).

shown in Figure 4, all three phenolic acids effectively inhibit lipase activity in a dose-dependent inhibition manner. Among those three phenolic acids, *p*-coumaric acid had the highest inhibitory effect compared to that of the other two phenolic acids when tested at low concentrations ($p < 0.05$). However, there was no significant difference between the inhibition rates of ferulic and *p*-coumaric acids at 200.0 or 250.0 μg/mL ($p > 0.05$), while the caffeic acid shows a slight but significantly lower inhibition rate than those of ferulic and *p*-coumaric acids ($p < 0.05$). The IC_{50} values of lipase inhibition of those phenolic acid standards were 251.2 ± 9.3, 219.3 ± 11.8, and 210.5 ± 6.9 μg/mL for caffeic, ferulic, and *p*-coumaric acids, respectively. *p*-Coumaric acid had the lowest IC_{50} value, while caffeic acid had the highest IC_{50} value, which also proved that *p*-coumaric acid was more effective than caffeic acid.

Phenolic acid Interactions on the Lipase Inhibitory Effect. It was shown that those three phenolic acids effectively inhibit lipase activity (Figure 4). However, the IC_{50} values of *A. oryzae* var. *effuses*-fermented oats and *A. oryzae*-fermented oats for inhibition were 127.7 and 132.1 μg gallic acid equivalents (GAE)/mL, respectively. None of the inhibition rates of the three phenolic acids were more than 30%, even at 150 μg/mL, so these three phenolic acids may have a synergistic effect in combination. Therefore, the inhibitory effect of binary combinations of those phenolic acids on lipase activity was examined, and three concentrations were selected near the IC_{50} values of the EA subfraction of fermented oats. The results are summarized in Tables 3−5, where the remnant lipase activity observed (V_{ab}) for phenolic acids was compared to the remnant lipase activity expected (V_c).

The data in Table 3 represent the interactions of caffeic and ferulic acids in combination at various concentrations. These data show that the combinations of caffeic and ferulic acid demonstrated synergy on inhibiting lipase activity, especially the combination of both phenolic acids at high concentrations. For example, when caffeic and ferulic acid were added alone at 150 μg/mL, the remnant lipase activity fractions were 0.80 and 0.74, respectively, and therefore, the V_c was 0.59. However, the observed remnant lipase activity fraction (V_{ab}) was 0.13 when caffeic and ferulic acids were added together. Therefore, the interaction $V_{ab} - V_c = -0.46$ demonstrated that those two phenolic acids had a significant synergistic effect on the inhibition of lipase activity at 150.0 μg/mL. Interestingly, the interaction was more often additive when at least one of those two phenolic acids was at a lower concentration (50.0 μg/mL).

The results of interactions between caffeic and *p*-coumaric acids on inhibiting lipase activity at different concentrations, which are summarized in Table 4, show similar behaviors when compared to those observed between caffeic and ferulic acids. The binary combinations of caffeic and *p*-coumaric acid showed strong synergeic effects on inhibiting lipase activity when both of them were at high concentrations. The strongest synergistic effect was found when both of those phenolic acids were at 150.0 μg/mL (Table 4). However, when one of the two phenolic acids was at 50.0 μg/mL, the interactions were additive. The same phenomena were also observed in the interactions between ferulic and *p*-coumaric acids (Table 5).

DISCUSSION

The expansion of adipose tissue with triglyceride (TG) accumulation is the main characteristic of obesity.[19] Most TG in adipocytes is synthesized from free fatty acids (FFAs), and dietary lipids represent the major source of FFAs and unwanted energy.[20,21] Thus, reducing the TG synthesis in adipocytes by inhibiting digestion and absorption of dietary lipids, especially dietary TG, could serve as an effective strategy in the treatment of obesity.[20−22] In the gastrointestinal tract, dietary TG is mainly digested by pancreatic lipase, which is synthesized and secreted by the pancreas and responsible for the hydrolysis of 50−70% of total dietary fats.[23]

Among the natural products which have inhibitory effects on pancreatic lipase activity, bioactive compounds derived from food origins are gaining more interest due to their few side effects. In the present work, the inhibitory effects on pancreatic lipase of subfractions from nonfermented and fermented oats were evaluated. The present study shows that the EA subfraction of nonfermented or fermented oats is more

Table 3. Interaction Effects of Caffeic Acid and Ferulic Acid on the Inhibition of Lipase Activity at Different Concentrations[a]

	50 μg/mL ferulic acid			100 μg/mL ferulic acid			150 μg/mL ferulic acid		
	value		interaction	value		interaction	value		interaction
	obsd (V_{ab})	expected (V_c)	$V_{ab} - V_c$	obsd (V_{ab})	expected (V_c)	$V_{ab} - V_c$	obsd (V_{ab})	expected (V_c)	$V_{ab} - V_c$
50 μg/mL caffeic acid	0.98	0.91	0.07AD[b]	0.96	0.86	0.10AD	0.68	0.73	−0.05AD
100 μg/mL caffeic acid	0.86	0.83	0.03AD	0.64	0.78	−0.14SY[c]	0.37	0.67	−0.30SY
150 μg/mL caffeic acid	0.62	0.74	−0.12 SY	0.39	0.69	−0.30SY	0.09	0.59	−0.50SY

[a]Values are the means of triplicate assays. [b]AD = additive interaction. [c]SY = synergistic interaction.

Table 4. Interaction Effects of Caffeic Acid and *p*-Coumaric Acid on the Inhibition of Lipase Activity at Different Concentrations[a]

	50 μg/mL caffeic acid			100 μg/mL caffeic acid			150 μg/mL caffeic acid		
	value		interaction	value		interaction	value		interaction
	obsd (V_{ab})	expected (V_c)	$V_{ab} - V_c$	obsd (V_{ab})	expected (V_c)	$V_{ab} - V_c$	obsd (V_{ab})	expected (V_c)	$V_{ab} - V_c$
50 μg/mL *p*-coumaric acid	0.95	0.86	0.09AD[b]	0.85	0.78	0.07AD	0.66	0.69	−0.03AD
100 μg/mL *p*-coumaric acid	0.82	0.79	0.03AD	0.61	0.72	−0.11SY[c]	0.35	0.63	−0.28SY
150 μg/mL *p*-coumaric acid	0.73	0.69	0.04AD	0.41	0.62	−0.21SY	0.08	0.55	−0.47SY

[a]Values are the means of triplicate assays. [b]AD = additive interaction. [c]SY = synergistic interaction.

Table 5. The Interaction Effects of Ferulic Acid and *p*-Coumaric Acid on the Inhibition of Lipase Activity at Different Concentrations[a]

	50 μg/mL ferulic acid			100 μg/mL ferulic acid			150 μg/mL ferulic acid		
	value		interaction	value		interaction	value		interaction
	obsd (V_{ab})	expected (V_c)	$V_{ab} - V_c$	obsd (V_{ab})	expected (V_c)	$V_{ab} - V_c$	obsd (V_{ab})	expected (V_c)	$V_{ab} - V_c$
50 μg/mL *p*-coumaric acid	0.88	0.79	0.09AD[b]	0.82	0.74	0.08AD	0.59	0.64	−0.05AD
100 μg/mL *p*-coumaric acid	0.78	0.73	0.05AD	0.57	0.68	−0.11SY[c]	0.34	0.59	−0.25SY
150 μg/mL *p*-coumaric acid	0.74	0.64	0.10AD	0.37	0.60	−0.23SY	0.06	0.51	−0.45SY

[a]Values are the means of triplicate assays. [b]AD = additive interaction. [c]SY = synergistic interaction.

effective in inhibiting pancreatic lipase compared to the other three subfractions evaluated. Moreover, the EA subfraction of each of the two fermented oats has a higher lipase inhibition rate than that of nonfermented oats at the same concentration. According to our previous study [14] and the current data, the TPC values of each subfraction of nonfermented and fermented oats are different from each other and show a high positive correlation with the lipase inhibition rate ($r = 0.921$, $p < 0.01$) (Figure 1). Therefore, the polyphenols in these subfractions may be the primary compounds reponsible for inhibiting lipase activity. Evidence from the present work also indicates that fungal fermentation can be used as an effective method to enhance the pancreatic lipase inhibitory effect of oats by increasing the TPC as well.

The inhibitory effects on pancreatic lipase activity of polyphenol-rich extracts have been reported in previous studies. For example, grape seed extract (GSE), enriched in polyphenols, has an inhibitory effect on lipoprotein lipase and pancreatic lipase, indicating that GSE has potential as a treatment method to reduce dietary fat absorption and slow the accumulation of fat in adipose tissue.[10] In China, tea is a popular dietary drink. Studies have found that white tea and green tea show effective inhibitory activity on pancreatic lipase, and the IC_{50} values were 22.0 and 53.0 μg GAE/mL, respectively.[9] In the present work, the IC_{50} values of *A. oryzae* var. *effuses*-fermented oats and *A. oryzae*-fermented oats for inhibition were 127.7 and 132.1 μg GAE/mL, respectively. The IC_{50} values indicate that white tea and green tea are likely more efficient in inhibiting pancreatic lipase activity. However, compared to tea, oats are used as a staple food in many areas of the world. Therefore, the amount of dietary intake of oats can be more than that of tea. Meanwhile, oats contain a high content of dietary fiber (DF), which also contributes to weight control, lowering blood cholesterol levels, preventing type 2 diabetes, and many other physiologic effects.[24,25] Therefore, fungi-fermented oats may provide a superior dietary therapy for treating obesity.

Orlistat is one of two clinically approved drugs for treating obesity by the FDA. The inhibitory effect of orlistat on lipase activity was also tested in the present work in vitro. It was found that the IC_{50} of orlistat was about 29.3 μg/mL in vitro, which was much lower than those of extracts from fermented oats. The recommended dose of orlistat is one 120 mg capsule three times daily. On the basis of the fact that the extraction yield of the EA subfraction was about 1.9%, we calculated that about 250 g of fermented oats, which can be consumed in three portions a day, may have the same inhibitory effect as that of 120 mg of orlistat. Such a dosage of fermented oats can be achieved if fermented oats are used as a routine staple food. However, it was also found that other subfractions besides the EA subfraction of fermented oats had some inhibitory effect on lipase activity as well. Moreover, when the effect of dietary fiber, which is high in fermented oats, on lipid intake reduction is considered, the actual demand of fermented oats may be far below the above calculated intake. However, such a conclusion needs to be proved by extra experiments in vivo. Currently, the fermented oats are recommended to be used as an alternative dietary intervention therapy of obesity control in assisting the obese medication treatment.

On the basis of our previous study,[14] *p*-coumaric, caffeic, and ferulic acids increased significantly after fermentation. The in vitro inhibition effects of those three phenolic acids on pancreatic lipase were also tested in this work. Lipase inhibitory effects of both *p*-coumaric and ferulic acids were significantly stronger than that of caffeic acid ($p < 0.05$). However, Karamac and Amarowicz[26] found that caffeic and ferulic acids showed stronger pancreatic lipase inhibitory activities than *p*-coumaric acid. The discrepancy between the present work and that of Karamac et al.[26] may be due to the differently produced batch of the lipase or the test conditions. The enzyme unit varied with the produced batch of the lipase. The inhibitory activities of EA subfractions of fermented oats may be attributed to the bioactivities of those three phenolic acids. In a previous study it was reported that a peanut shell extract (PSE) which contained caffeic, ferulic, and benzoic acids also possessed a pancreatic lipase inhibitory effect.[27]

Earlier studies showed that there were synergistic effects when two or more polyphenols were combined.[10,16] The inhibitory effect of GSE on lipase was also assumed to be caused by a synergistic effect, since GSE had many different

bioactive compounds such as flavonoids and procyanidins.[10,23] The EA subfractions of nonfermented and fermented oats used in the present work contained many different phenolic acids as well.[14] Therefore, the inhibitory effects of all EA subfractions on lipase activity may be due to the synergistic action of those compounds. To prove such synergistic effects, we tested the lipase inhibition interactions of each binary combination of *p*-coumaric, ferulic, and caffeic acid standards, which were the primary phenolic acids within the EA subfractions. Synergism was observed, especially at high concentration. However, when tested at low concentrations, the interactions of binary combinations of those phenolic acids were additive rather than synergistic. Therefore, those three phenolic acids more likely contribute additive effects on the lipase inhibition in the fermented oat matrix with each other, because their contents were lower than those in the binary combinations that showed synergistic actions.[14] However, due to the interaction complications of compounds, those phenolic acids may have a synergistic interaction when they are contained in a sample with many other phenolic acids, even though at a low concentration. The lipase inhibition of the EA subfractions in the present work may be due to the combined actions of the various phenolic acids in the extracts (a positive correlation between the TPC and inhibition rate, $r = 0.921$, $p < 0.01$). Generally, the present work has confirmed that there is a possibility of synergistic interactions of those phenolic acids in natural extracts.

The results of the present work indicate that fungi-fermented oats may have an inhibitory effect on pancreatic lipase and phenolic acids in fermented oats may be primarily responsible for this bioactivity. In terms of inhibition, the phenolic acids in fermented oats may have a synergistic effect. Therefore, fungi-fermented oats and foods rich in polyphenols could be recommended as a complementary therapy for treating obesity.

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Notes

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