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Full Paper

Inhibition of α-amylase, α-glucosidase and pancreatic lipase activities in vitro by sacha inchi (*Plukenetia volubilis* L.) protein hydrolysates and their fractionated peptides

Saranya Suwanangul [1], Monisola A. Alashi [2], Rotimi E. Aluko [2], Worasit Tochampa [1] and Khanitta Ruttarattanamongkol [1,*]

[1] Department of Agro-Industry, Faculty of Agriculture, Natural Resources and Environment, Naresuan University, Phitsanulok, 65000, Thailand
[2] Department of Food and Human Nutritional Sciences, University of Manitoba, Winnipeg, R3T 2N2, Canada
* Corresponding author, e-mail: khanittar@nu.ac.th

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Abstract: Protein hydrolysates were produced via enzymatic hydrolysis of sacha inchi protein isolate using three different protease enzymes: pepsin, papain and Flavourzyme. Each hydrolysate sample was then fractionated by ultrafiltration membrane at various molecular weight (MW) cut-off ranging from 1-10 kDa. The results show that peptide fraction with MW of 1-3 kDa produced with pepsin had the highest inhibitory activity against α-amylase. Peptide fraction with MW < 1 kDa produced by Flavourzyme had the highest inhibitory activity against α-glucosidase (51.06±0.08%), followed by fractions with MW < 1 kDa and 1-3 kDa (48.21±0.12% and 48.27±0.17%) produced by pepsin, and unfractionated hydrolysate produced by papain (42.81±0.38%). However, the fractionated peptides had reduced capacity in inhibiting lipase activity (IC_{50}>3.0 mg/mL) compared with the unfractionated counterparts (IC_{50}<3.0 mg/mL). The findings suggest that sacha inchi protein-derived peptides have the potential for being utilised as functional foods as they can play an important role in the management of caloric intake.

Keywords: sacha inchi, *Plukenetia volubilis*, enzyme inhibition, protein hydrolysates, α-amylase, α-glucosidase, pancreatic lipase

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INTRODUCTION

One approach to treating the conditions of obesity and type-2 diabetes mellitus (T2DM) is the inhibition of digestive enzymes. Synthetic compounds such as Acarbose and Orlistat that can delay carbohydrate digestion and reduce the subsequent intestinal glucose absorption rate, and Miglitol that acts as a reversible inhibitor of α-glucosidase enzymes in the small intestine [1, 2], have been developed for enzyme inhibition purposes. However, these compounds tend to possess negative side effects ranging from diarrhea to hepatotoxicity. Both obesity and T2DM have been identified as public health concerns and with the global prevalence of both conditions, the identification of alternative enzyme inhibitors becomes imperative [3]. Compounds from natural sources such as dietary components are more desirable as they are thought to possess a lower risk of negative side effects on human health when compared to synthetic inhibitors.

A range of natural compounds and foods have been investigated and shown to possess inhibitory activities against gastrointestinal enzymes [3]. Examples include potato fruit water and pulses (pea, chickpea and lentil) [4-6]. This can be achieved by the inhibition of the key enzymes involved in food digestion. Chickpea extract, for example, was found to inhibit the digestive enzymes involved in carbohydrate and lipid metabolism, i.e. α-amylase (72.6%), α-glucosidase (10.9%) and lipase (85.4%) [7]. The hydrolysates produced by Protamex from *Bacillus* sp. (EC 3.4.21.14; ≥16 U/g) were subjected to ultrafiltration to separate peptides into fractions of different molecular weights. Six fractions were produced and tested for the inhibitory activity of α-amylase. Fractions with molecular weight (MW) lower than 3 kDa had the highest inhibitory activity (62.10%) against α-amylase. Similarly, peptides present in rice bran hydrolysates with MW <3 kDa had the highest inhibitory activity (47.9%) against α-glucosidase [8]. Uraipong and Zhao [9] have shown that bioactive peptides possess significant inhibitory activities against gastrointestinal enzymes. When the amount of bioactive peptides that can be safely consumed is taken into consideration, these peptides have the potential to be developed into non-drug supplements for the management of obesity as well as T2DM [9].

It has been established that the use of dietary modification or nutritional intervention in the prevention and/or management of obesity and T2DM in order to promote weight loss is an important strategy [10]. This strategy includes suppressing the digestion of carbohydrates in the gut by inhibiting carbohydrate-hydrolysing enzymes such as α-amylase and α-glucosidase, thus reducing the amount of glucose absorbed into the body. In addition, inhibition of pancreatic lipase reduces the digestion of triacylglycerides and in turn the efficiency of fat absorption in the small intestine, thereby initiating modest long-term reduction in body weight [11].

Sacha inchi (*Plukenetia volubilis* L.) is native to the Peruvian Amazon and its edible dark brown seeds are very rich in oil (35-60%), protein (27%) with essential amino acids such as cysteine, tyrosine, threonine and tryptophan, carbohydrate (in the form of amylose starch), fibre, vitamin E, polyphenols, minerals and others [12]. The protein content of Sacha inchi seeds is higher than that of chickpea seed (19%), sunflower seeds (17.4%), flax seeds (17.8%) and pumpkin seeds (21.2%) [13-15]. Sacha inchi thus has excellent potential for being used in protein concentrate production since it contains a high amount of protein. Moreover, the derived protein concentrate can also be used for producing protein hydrolysate which, due to

its high level of essential amino acids, has a major effect on biological activities [16]. Protein hydrolysate is obtained from the breakdown of protein by enzymes into small fractions that can be easily absorbed in the human body [16]. Diets relatively high in protein (18-30%) are effective in the management of obesity due to their suppression of appetite [17] and can reduce postprandial blood glucose in both healthy individuals and patients with impaired glucose metabolism [18]. High-protein diets have been proved to reduce weight and suppress insulin response [19], but their long-term effects are unknown [20]. A protein preload before a meal has been shown to be effective in lowering the postprandial glycemic response both in T2DM patients and healthy subjects [21]. The sacha inchi protein hydrolysates produced by Calotropis proteases has increased antioxidant activities compared with sacha inchi protein concentrate [22].

Peru has exported sacha inchi oil (Agraria) as the main Sacha inchi product (approximately 98%). Other products from sacha inchi are seeds or other derivatives [23]. In 2017 around 271 acres of Sacha inchi were cultivated in Thailand and around 80% of exported sacha inchi products were sacha inchi seed oil [24]. Sacha inchi seeds are normally used for the extraction of oil owing to their high oil content (~50%). The main by-product of the oil production is the seed residue or meal, which contains more than 50% protein and is normally discarded as waste or only used as animal feed. Based on the nutritive values in the sacha inchi residue, it has the potential for being developed into protein-derived peptides and used as inhibitors of α-amylase, α-glucosidase and pancreatic lipase activities. To the best of our knowledge, information is scarce on the inhibitory properties of sacha inchi protein hydrolysate and its derived peptides against gastrointestinal enzymes. The purpose of this study is to evaluate the in-vitro inhibitory activities of sacha inchi protein hydrolysates (unfractionated) produced by different protease enzymes and also of their fractionated peptides produced by ultrafiltration membrane against α-amylase, α-glucosidase and pancreatic lipase.

MATERIALS AND METHODS

Materials and Chemicals

Sacha inchi meal, a by-product of oil extraction, was kindly provided by Tai.C.M.S. Standard Industrial Co. (Chiang Rai province, Thailand). Three different protease enzymes were used, viz. pepsin, papain and Flavourzyme. Pepsin from porcine gastric mucosa (EC 3.4.23.1; ≥250 units/mg solid), papain from papaya latex (EC 3.4.22.2; 10 units/mg protein), Flavourzyme® from *Aspergillus oryzae* (EC 232-752-2; ≥500 U/g), porcine pancreatic α-amylase, porcine pancreatic lipase, rat intestinal acetone powder, acarbose, orlistat, 4-nitrophenyl α-D-glucopyranoside (PNP-glycoside) and 4-methylumbelliferyl oleate (4-MU oleate) were purchased from Sigma-Aldrich (USA). Other chemical reagents were obtained from Fisher Scientific (Canada). All chemical reagents were of analytical grade and used without further purification, whereas distilled water was used for the preparation of reagents.

Preparation of Sacha Inchi Protein Isolate (SIPI)

The SIPI was produced according to the method described by Adebowale et al. [25]. A 100 g of sacha inchi meal was dispersed in deionised water (1 L) and the mixture adjusted to pH 11.5 with 2M NaOH to solubilise proteins. The resultant dispersion was stirred at 60°C for

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1 hr followed by centrifugation (7000 x g, 30 min. at 4°C). The supernatant was adjusted to pH 7.0 with 2M HCl to precipitate most of the proteins. Thereafter, the mixture was centrifuged (7000 x g, 30 min. at 4°C) and the resultant precipitate was freeze-dried to produce SIPI.

Preparation of Sacha Inchi Protein Hydrolysates

Enzymatic hydrolysis of SIPI was carried out using each different protease enzymes (pepsin, papain and Flavourzyme). Briefly, A 100 g of SIPI was mixed with 1000 mL of distilled water. Under constant stirring, the beaker containing the protein solution was placed on a magnetic stirring hot plate equipped with external temperature and pH probes. The optimal pH of the protein solution was achieved using either 1 M NaOH or 1 M HCl. The optimal conditions for pepsin, papain and Flavourzyme were pH 2.0 at 37°C, pH 7.0 at 55°C and pH 6.5 at 50°C respectively [4, 8, 26]. The hydrolysis process was performed by adding each protease enzyme to the protein solution at 1% concentration (on the basis of protein content in the substrate, w/w). Once the enzyme was added, the temperature and pH were maintained and monitored for 4 hr as described above, after which hydrolysis was terminated by adjusting to pH 7.0 with either 1M NaOH or 1M HCl followed by heating at 95°C for 15 min. to ensure complete denaturation of the enzymes. The reaction mixture was then cooled to room temperature and centrifuged at 10,000 x g for 30 min. at 4°C using an Allegra^{TM} 6R centrifuge (Biotech Equipment Sales, USA) to separate the soluble hydrolysed material (peptides) from the unhydrolysed residue (mainly undigested protein). The clear supernatant was collected as the hydrolysate and a portion freeze-dried and stored at -20°C until further analysis.

Preparation of Peptide Fractions

The collected supernatant was successively passed through an Amicon stirred ultrafiltration cell (Merck KGaA, Germany) using 1 kDa, 3 kDa, 5 kDa and 10 kDa molecular weight (MW) cut-off membranes (MWCO) (Merck KGaA, Germany) to produce peptides of different sizes. The supernatant was first passed through the 1-kDa membrane; the permeate (<1 kDa) was collected. The retentate was mixed with an equal volume of distilled water and passed through a 3-kDa membrane; the permeate (1-3 kDa) was collected. This process was repeated by passing the 3-kDa retentate through a 5-kDa membrane to collect the permeate (3-5 kDa), whereas the 5-kDa retentate was passed through a 10-kDa membrane and the permeate (5-10 kDa) was collected. The resulting permeates and 10-kDa retentate were freeze-dried and stored at -20°C. The freeze-dried hydrolysates, membrane fractions and retentate were weighed and analysed for their peptide content using the modified Lowry method [27]. The per cent yield of protein hydrolysate was determined as the ratio of peptide weight of each protein hydrolysate to the protein weight of SIPI. Similarly, the per cent yield of each ultrafiltration membrane fraction was calculated as the ratio of peptide weight of each fraction to peptide weight of each protein hydrolysate before fractionation. The formula is as follows:

Peptide yield of protein hydrolysate or fractionated peptide (%) =
(Peptide content of hydrolysate or peptide fraction / initial protein weight of SIPI or initial peptide weight of hydrolysate) x 100% (1)

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Inhibition of α-Amylase Activity

The inhibition of α-amylase activity was assayed using soluble starch as substrate according to the procedure described previously by Siow et al. [28] with some modifications. Freeze-dried samples were dissolved in 1 mL of 0.02 M sodium phosphate buffer (pH 6.9) containing 0.006 M NaCl. A 100-µL aliquot of each sample (final peptide concentration = 0.5, 1.0, 1.5 and 2.0 mg/mL) and 100 µL of α-amylase solution (~30 U/mg; final concentration = 1.0 mg/mL) were added in test tubes and allowed to incubate for 10 min. at 25°C. After incubation, 100-µL of 1% starch solution (dissolved in the above buffer) was added and incubated at 25°C for 10 min. The reaction was terminated by adding 200 µL of dinitrosalicylic acid (DNS) colour reagent (96 mM DNS, 2M sodium potassium tartrate tetrahydrate and 2M NaOH) followed by incubation in a boiling water bath at 100°C for 5 min. The reaction mixture was allowed to cool to room temperature, after which 3 mL of doubly-distilled water was added. A 200-µL aliquot of the reaction mixture was then transferred to a 96-well microplate and the absorbance read at 540 nm using a Synergy™ H4 Hybrid microplate reader (BioTek Instruments, USA) set at 25°C. A blank reading (with buffer added to replace enzyme) was subtracted from each well. The enzyme activity was quantified by measuring the maltose equivalents released from starch at 540 nm. The pharmacological α-amylase inhibitor acarbose was similarly assayed and used as a positive control. The extent of α-amylase inhibitory activity was calculated as follows:

$$\text{Alpha-amylase inhibition (\%)} = [(Ac - (As - Asb)) / Ac] * 100\% \quad (2)$$

where Ac=absorbance of control, As=absorbance of sample and Asb=absorbance of sample blank.

Inhibition of α-Glucosidase Activity

The α-glucosidase inhibitory activity was assayed according to previously described methods [3] with some modifications. Briefly, 300 mg of rat intestinal acetone powder was homogenised in 9 mL of 0.9% NaCl solution and centrifuged at 12000 x g for 30 min. and the clear supernatant was used as the source of α-glucosidase enzyme. Freeze-dried protein hydrolysates were dissolved in 0.1 M sodium phosphate buffer (pH 6.9) and 50 µL (final peptide concentration = 0.5, 1.0, 1.5 and 2.0 mg/mL) were pre-mixed with 50 µL of the α-glucosidase enzyme (intestinal acetone powder from rat; final concentration = 8.33 mg/mL) in a 96-well microplate and incubated at 37°C for 10 min. Following incubation, 100 µL of 5 mM PNP-glycoside solution (in 0.1M sodium phosphate buffer, pH 6.9) were added to each well and absorbance read continuously at 405 nm for 30 min. (at every 30-sec. interval) using the Synergy™ H4 microplate reader set at 37°C. A blank reading (all reagents except the enzyme) was subtracted from each well. The α-glucosidase activity was quantified by measuring the absorption intensity of p-nitrophenol released from the PNP-glycoside at 405 nm. Acarbose was assayed using the same protocol and served as a positive control. The extent of α-glucosidase inhibitory activity was calculated as follows:

$$\text{Alpha-glucosidase inhibition (\%)} = [((Act - Actb) - (Asp - Aspb)) / [Act - Actb] * 100\% \quad (3)$$

where Act=absorbance of control, Actb=absorbance of control blank, Asp=absorbance of sample and Aspb=absorbance of sample blank.

Inhibition of Pancreatic Lipase Activity

Pancreatic lipase inhibitory activity was assayed employing the method described by Awosika and Aluko [3] with some modifications. The activity was determined by measuring the release of 4-methylumbelliferone (4-MU) from the substrate 4-MU oleate. A 25-µL aliquot of samples (final peptide concentration = 2.0, 4.0, 6.0, 8.0 and 10.0 mg/mL) dissolved in Tris buffer (13mM Tris-HCl, 150mM NaCl and 1.3mM $CaCl_2$, pH = 8) and 225 µL of a 0.5 mM 4-MU oleate solution was mixed in a 96-well microplate and incubated for 15 min. at 37°C. After incubation, 25 µL of pancreatic lipase solution (~50 units/mg solid; final concentration = 3.125 U/mL) were added to start the enzyme reaction and the mixture incubated at 37°C for 1 hr. After incubation, the amount of 4-MU released by the lipase was measured with a microplate reader at a wavelength of 400 nm. The pharmacological pancreatic lipase inhibitor (orlistat) was used as a positive control. The extent of pancreatic lipase inhibitory activity was calculated as follows:

$$\text{Lipase Inhibition (\%)} = [(Acn-Asa)/Acn] * 100\% \quad (4)$$

where Acn=absorbance of control and Asa=absorbance of sample.

The inhibitory concentration that reduces lipase activity by 50% (IC_{50}) for the protein hydrolysates and fractionated peptides was obtained by analysis of a plot of percentage inhibition vs sample concentration using GraphPad Prism version 6.0 (GraphPad Software, USA).

Statistical Analysis

The data were collected in triplicate and subjected to analysis of variance (ANOVA) using SPSS 11.0 software. Significant differences among means were differentiated by Duncan's new multiple range tests at a statistical significance of 95%

RESULTS AND DISCUSSION

Peptide Content and Percentage Peptide Yield

The peptide content and percentage of peptide yield of the protein hydrolysates and fractions after enzymatic hydrolysis are presented in Table 1. The peptide content of the protein hydrolysates produced by different proteases was not much different. Because of the differences in proteolytic specificity, there is a possibility that the hydrolysates may contain different types of peptides with varying inhibitory fraction activities against target enzymes. The peptide content of the hydrolysates and peptide fractions varied considerably, which could be attributed to differences in peptide cleavage specificity of the enzymes used in this work. The 3-5 kDa, 5-10 kDa and >10 kDa fractions had significantly ($p < 0.05$) higher peptide content than the <1 kDa and 1-3 kDa fractions. Initial passage through the 1-kDa and 3-kDa membranes would have removed most of the low MW non-peptide components such as salts and soluble sugars, which ended up in the permeates, leading to reduced peptide

content. In contrast, permeates from the 5-kDa and 10-kDa membranes would contain lower amounts of these low MW non-peptide components, resulting in a higher peptide content.

The result of the percentage peptide yield indicates the efficiency of the enzymatic hydrolysis process in liberating peptides from the substrate protein primary structure. A higher peptide yield reflects an increased protein breakdown and peptide release [29]. Amongst the three enzymes used for hydrolysis, pepsin produced a hydrolysate with a significantly *($p < 0.05$)* higher yield (78.3%) than that of papain and Flavourzyme. The presence of lower percentage peptide yield in the >10 kDa fraction also reflects the superior ability of pepsin to reduce the peptide size when compared with papain and Flavourzyme. The higher proteolytic efficiency of pepsin could be due to the fact that it has broad specificity [30], which enables the production of a wider variety and quantity of peptides when compared with papain and Flavourzyme.

Table 1. Percentage peptide content (PC) and percentage peptide yield (PY) of sacha inchi protein hydrolysates and fractionated peptides

Sample	Pepsin		Papain		Flavourzyme	
	PC	PY	PC	PY	PC	PY
Hydrolysate	90.95±0.66^{c}	78.3±0.13^{a}	90.43±1.13^{a}	65.50±0.08^{a}	90.16±1.54^{a}	65.10±0.06^{a}
<1 kDa	76.63±0.02^{e}	12.7±0.06^{e}	53.63±0.08^{d}	7.37±0.06^{e}	59.00±0.15^{c}	9.00±0.05^{e}
1-3 kDa	82.01±0.19^{d}	16.3±0.02^{c}	71.33±0.15^{c}	10.09±0.01^{d}	82.83±0.06^{b}	11.83±0.08^{d}
3-5 kDa	90.11±0.14^{c}	18.4±0.11^{b}	88.39±0.11^{b}	10.10±0.04^{d}	90.18±0.06^{a}	13.18±0.13^{c}
5-10 kDa	93.27±0.10^{b}	13.2±0.05^{e}	92.92±0.09^{a}	12.85±0.05^{c}	92.00±0.11^{a}	13.30±0.10^{c}
> 10 kDa	95.41±0.08^{a}	15.1±0.03^{d}	88.06±0.07^{b}	16.62±0.08^{b}	82.13±0.10^{b}	16.05±0.03^{b}

Note: Results are presented as mean ± SD (n = 3). For each column, mean values with different letters are significantly different at $p < 0.05$.

Alpha-Amylase Inhibition

One of the major enzymes involved in the digestion of dietary starch is α-amylase, which releases oligosaccharides that can be further broken down into glucose, which is rapidly absorbed by the body. Alpha-amylase activity inhibition is thus regarded as an effective strategy for managing diabetes [31]. The highest inhibitory activity of the 1-3 kDa fraction produced by pepsin against α-amylase was much lower in comparison to the standard acarbose (Figure 1A). This is not surprising since acarbose is a purified synthetic inhibitor of α-amylase, whereas the hydrolysates are mixtures of peptides and probably some non-peptide components. Within the pepsin group, the 1-3 kDa fraction was the most active with a significantly ($p < 0.05$) higher value than other fractions and the unfractionated hydrolysate at concentration of 2.0 mg/mL (Figure 1A). In the papain group at 2.0 mg/mL the unfractionated hydrolysate showed the highest inhibitory activity of 29.44±0.55%, while among the fractionated group, the 1-3 kDa fraction showed the highest inhibitory activity (Figure 1B). With regards to the Flavourzyme group, the 1-3 kDa fraction exhibited the highest inhibitory activity of 28.11±0.83%, while the unfractionated hydrolysate at 2.0 mg/mL gave 25.71±0.18% inhibitory activity (Figure 1C). The α-amylase inhibition values for all hydrolysates and peptide fractions remained unchanged at concentration > 2.0 mg/mL, which indicates a competitive type of

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inhibition. The competitive mode of inhibition exhibited by the hydrolysates and peptide fractions means that the samples interact with the active site of α-amylase and compete with the substrate for binding to the active site of α-amylase, thereby preventing the breakdown of starch into oligosaccharides, disaccharides and ultimately glucose [2].

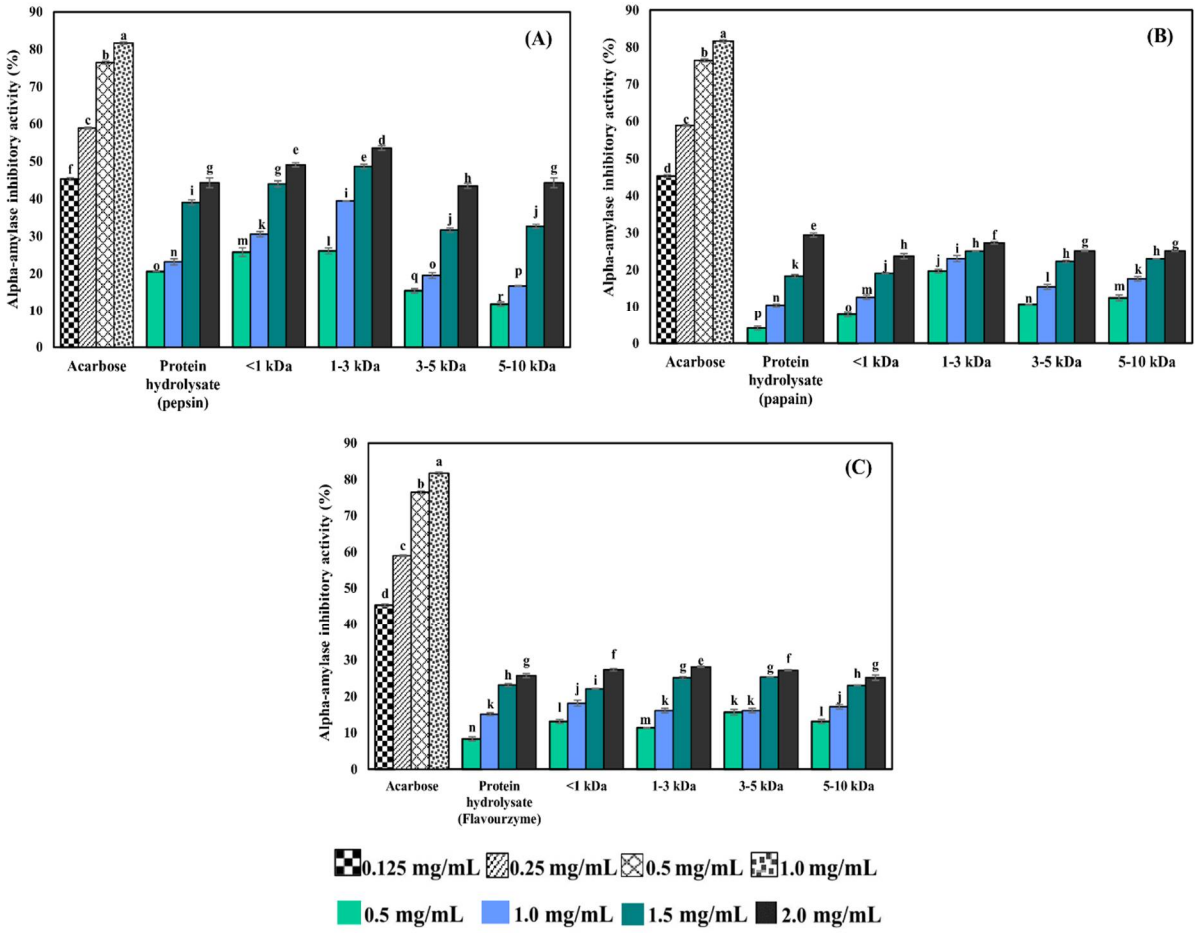

Figure 1. Alpha-amylase inhibitory activity of sacha inchi protein hydrolysates and fractionated peptides by (A) pepsin, (B) papain and (C) Flavourzyme. Results are presented as mean ± standard deviation (n=3). For each figure, bars with different letters have mean values that are significantly different at $p < 0.05$.

The wide variation in the potency of the samples against α-amylase probably reflects the differences in peptide composition. The results indicate that fractionation improves the α-amylase inhibitory activity of the pepsin and Flavourzyme hydrolysates (but not the papain samples). A possible reason may be the fact that smaller and narrower-sized peptides can easily bind to the active site of the enzyme, resulting in a higher inhibitory activity. Conversely, the unfractionated hydrolysate contains a larger and wider range of peptides, meaning it will have weaker enzyme binding ability as previously suggested for other protein hydrolysates [26]. Another possible explanation is that the unfractionated hydrolysates contain certain peptides which may possess antagonistic effects against the inhibitory action of the active peptides [32]. The results are consistent with other studies which reported that low MW peptides (specifically

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<1 and <3 kDa) from Pinto beans had the highest α-amylase inhibitory activity of 49.9% and 62.1% respectively, among all protein hydrolysates [33].

Alpha-Glucosidase Inhibition

As an important enzyme in starch hydrolysis, α-glucosidase is located in the epithelium of the small intestine. It is involved in starch digestion through the breakdown of oligo- and di-saccharides to produce glucose, which is then absorbed by the body. Therefore, the inhibition of this enzyme is another effective strategy for lowering serum glucose levels and, ultimately, alleviating the disease symptoms associated with diabetes [26]. In our study a dose-dependent effect was noted as the inhibitory activities of the samples increased with increasing concentration of less than 2.0 mg/mL (Figure 2). The results of α-glucosidase inhibition are similar to those of α-amylase inhibition as all samples reduced α-glucosidase activity through a competitive type of inhibition. The competitive mode of inhibition exhibited by the peptides indicates that the peptides interacted with the active site of α-glucosidase and competed with the substrate for binding to the active site of α-glucosidase, thereby retarding the conversion of disaccharides to monosaccharides [3].

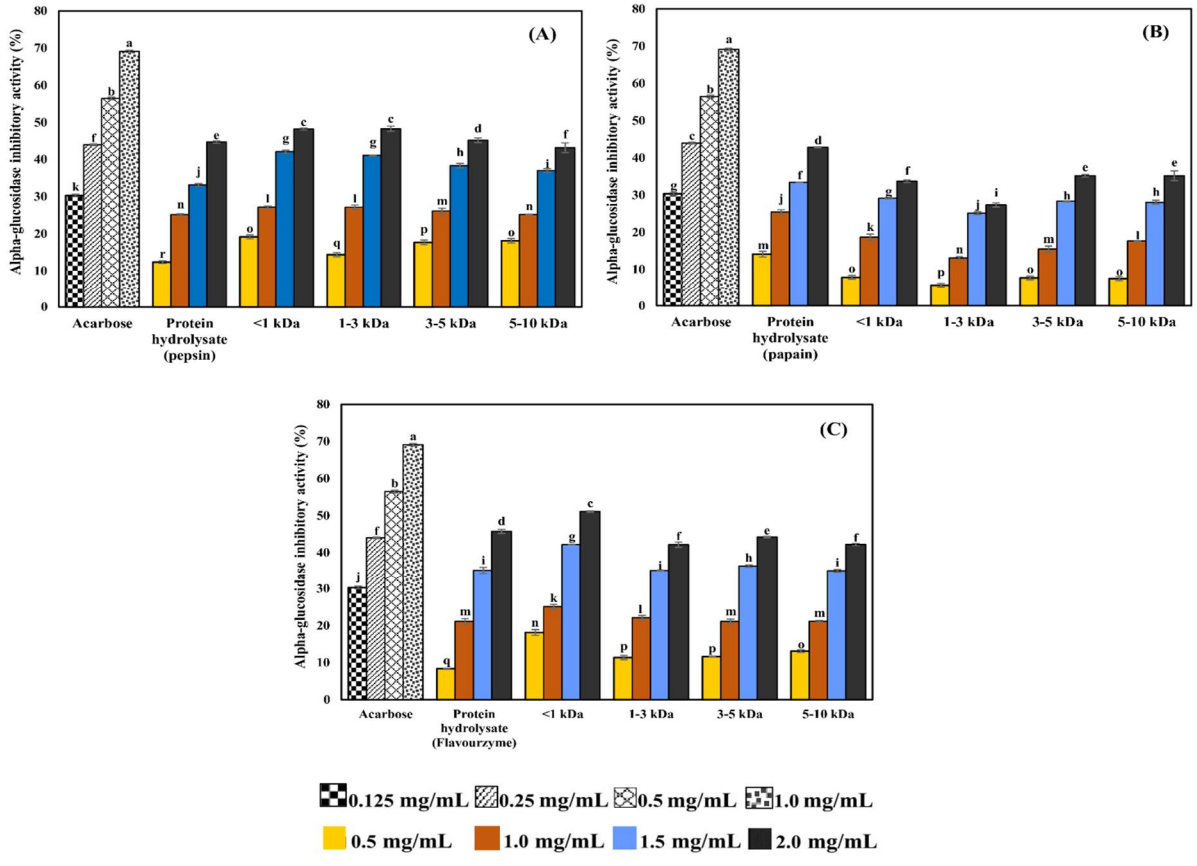

Figure 2. Alpha-glucosidase inhibitory activity of sacha inchi protein hydrolysates and fractionated peptides by (A) pepsin, (B) papain and (C) Flavourzyme. Results are presented as mean ± standard deviation (n=3). For each figure, bars with different letters have mean values that are significantly different at $p < 0.05$.

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Based on the highest concentration tested (2.0 mg/mL), the <1 kDa fraction by Flavourzyme had the highest inhibitory activity (Figure 2C). Within the papain group, the unfractionated hydrolysate had a higher inhibitory activity when compared with the peptide fractions, whereas for fractionated group the 3-5 kDa and 5-10 kDa fractions showed the highest inhibitory activities (Figure 2B). For the pepsin group, the inhibitory activities of the fractionated peptide fractions were higher than that of the unfractionated hydrolysate, with the <1 kDa and 1-3 kDa fractions exhibiting the highest activities (Figure 2A). The results suggest that synergistic effects are operating for the stronger α-glucosidase inhibitory activity of papain hydrolysates; peptide fractionations leads to reduction of these effects. In contrast, it is possible that pepsin and Flavourzyme hydrolysates contain peptides with antagonistic effects, which are removed after peptide fractionation. Some studies have reported that low MW peptides are also strong inhibitors of α-glucosidase activity. For example, the <1 kDa peptide fraction derived from Pinto Durango bean exhibits an inhibitory activity of 76.4% against α-glucosidase [34], whereas the value is 47.9% for the <3 kDa peptide fraction from rice bran against α-glucosidase [9] and 53.4% for the <1 kDa peptide fraction from yellow field pea against α-glucosidase [3].

Pancreatic Lipase Inhibition

Pancreatic lipase is a key enzyme in charge of intestinal digestion of dietary triacylglycerols, a major source of excess calorie intake. The inhibition of pancreatic lipase causes a reduction in the efficiency of fat absorption in the small intestine, in turn leading to modest but long-term reduction in body weight [35]. Therefore, the suppression or delay of triacylglycerol digestion and absorption through the inhibition of lipase activity has been targeted for the development of anti-obesity agents [36]. Unlike α-amylase and α-glucosidase inhibition, more than 50% reduction in lipase activity was achieved. The inhibition values indicate that the peptides exert lipase inhibition activity through a non-competitive type of inhibition. This is because the inhibition values decrease with increasing concentrations of the hydrolysates and fractionated peptides. The non-competitive mode indicates that the hydrolysates and peptide fractions did not compete with the substrate for binding to the active site.

The IC_{50} value is defined as the concentration of an inhibitor that is required for 50% inhibition of the enzyme activity and it is commonly used for the measurement of inhibitor potency. It has been established that a lower IC_{50} value indicates a higher inhibitory activity. The results show that the unfractionated hydrolysates have lower IC_{50} values (<3.2 mg/mL) and are more potent lipase inhibitors when compared with the fractionated peptides, which have >3.2 mg/mL values (Figure 3). The pepsin (2.57±0.5 mg/mL) and Flavourzyme (2.98±0.4 mg/mL) hydrolysate had lower IC_{50} values than the papain hydrolysate indicating higher potency with respect to lipase activity inhibition. Ultrafiltration separation into different peptide sizes all led to decreased inhibitory potency against lipase. This might be because of the synergistic effect of the peptides contained in the unfractionated hydrolysates, which was reduced or lost during the fractionation process. On a similar note, Awosika and Aluko [3] also observed that yellow field pea inhibited lipase activity at low IC_{50} values (<5 mg/mL). Similarly, a study by Lee et al. [37] reported that legume seed extracts had IC_{50} values between 6.0-8.0 mg/mL. According to Ngoh et al. [38], synthesized sequences based on Pinto bean peptides inhibited lipase activity at levels ranging from ~23% to 87%.

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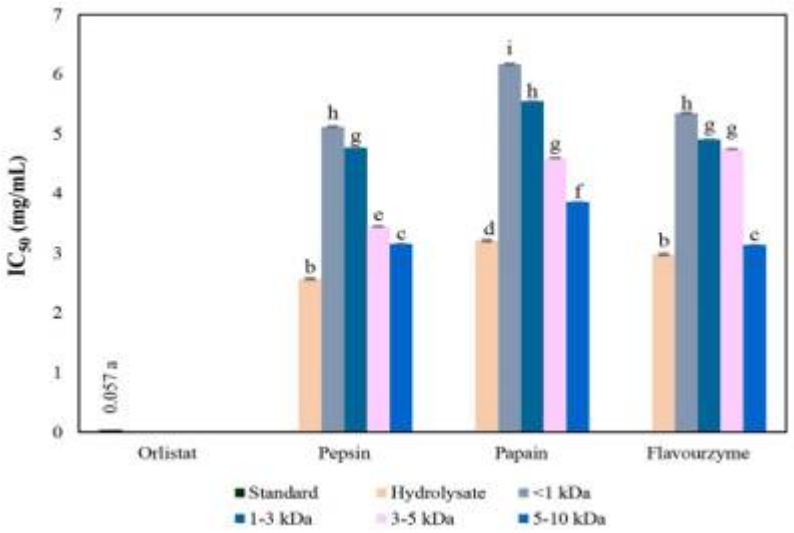

Figure 3. IC_{50} of sacha inchi protein hydrolysates and fractionated peptides produced by different enzymes. Results are presented as mean ± standard deviation (n=3). Bars with different letters have mean values that are significantly different at $p < 0.05$.

CONCLUSIONS

Based on our findings, sacha inchi protein hydrolysates and their fractionated peptides have the potential to inhibit key digestive enzymes. The results could be useful for a dietary approach to calorie management. However, additional experiments on the inhibitory activity of the hydrolysates and peptide fractions against digestive enzymes in vivo as well as the ability of the hydrolysates and peptides to produce weight loss and the safety aspects are needed in order to make commercial uses of these peptides in the future.

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