



Extraction and characterization of pectin from apple pomace and its evaluation as lipase (steapsin) inhibitor

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ABSTRACT

Pectin extracted from the apple pomace was evaluated for the *in vitro* inhibition of pancreatic lipase (steapsin). Pectin was extracted from two different varieties of apples, i.e., *Malus pumila* and *Spondias dulcis* using two extractants, i.e., hydrochloric acid and citric acid (CA), separately at pH 2.5. The effect of the extraction process on the structure of the extracted pectin was evaluated by the physico-chemical parameters and different techniques such as XRD, ^{13}C NMR, FTIR or Raman spectroscopy. The lipase inhibition was observed to be dependent both on the source as well as the extractant process used. The maximum lipase inhibition (94.30%) was obtained with the pectin extracted from *Malus pumila* by CA process, which is comparable to that of the commercial pectin, i.e., 94.15%. Tetrahydrolipstatin was used as reference steapsin inhibitor. Therefore, the extracted pectin has potential use in the anti-obesity formulations and other applications like personal care products.

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1. Introduction

Human obesity is one of the most serious health problems. Obesity is associated with an increased risk of several serious diseases including hypertension, coronary heart disease, type II diabetes, stroke, osteoarthritis and cancer. Consumption of the dietary fat is an important contributor to human obesity. Gastric and pancreatic lipases are the principal lipolytic enzymes in the gastrointestinal (GI) tract that are responsible for the hydrolysis of triacyl glycerides (TAG). Thus, inhibition of lipase in the GI tract reduces the amount of fat that can be absorbed. The inactivation of pancreatic lipase by diethyl *p*-nitrophenyl phosphate (Moreau, Moulin, Gargouri, Noel, & Verger, 1991), 5-(dodecylthio)-2-nitrobenzoic acid (Cudrey, Tilbeurgh, Gargouri, & Verger, 1993), carbamates (Lina, Laia, Tsaia, Hsiehb, & Tsai, 2006), and with the synthetic drugs like tetrahydrolipstatin (THL, Orlistat) (Tissa, Lengsfeldb, Carrière, & Verger, 2009), has been reported. Apart from the synthetic drugs, many polymers of natural origin have also been reported as lipase inhibitors, and these include wheat bran (Isaksson, Lundquist, & Ihse, 1982), poly(dextrose) (Ogata et al., 1997) and poly(lysine) (Tsujita et al., 2003). Flavan dimers isolated from the fruits of *Cassia nomame* have also been reported to show lipase-inhibiting effects (Hatano et al., 1997). Lipase inhibitors were isolated from the sunflower seed (*Helianthus annuus* L.) by ammonium sulphate fractionation (Chapman, 1987). Anti-obesity drug orlistat also acts

via lipase inhibition, but it exhibits many side effects. On the other hand, the functional food materials like fibers or the biopolymers are not only safe to use, these are also very effective. The use of polymer drug is far effective than its small molecule analogue due to the high local concentration of the active sites on the polymer chains (Phan & Tso, 2001).

In view of the above, we investigated the effect of pectin extracted from two different varieties of apple as lipase inhibitor. The fruit wastes from the agro-industries consist of the cull fruits and mechanically damaged fruits along with peel, core and pomace of the processed fruits. Pectin is one of the important biopolymer recovered primarily from the citrus and apple wastes. The extraction of pectin from the fruit waste has been reported using various techniques. Its extraction from the apple pomace (Jain, Ghankrokta, & Agrawal, 1984) or peel waste has been reported (Virk & Soghi, 2004). The effect of the extraction conditions on the yield and purity of the apple pomace pectin has also been reported (Haikel et al., 2007). Hydrolysis of the apple pectin by the coordinated activity of pectic enzymes has also been reported (Nikolic & Mojovic, 2007). Wang et al. (2007) reported conditions for the optimization of the microwave assisted pectin extraction process from the apple pomace using response surface methodology. The process and the property-profile of the pectin extracted from apple have been described in the literature (Joye & Luzio, 2000; Kratchanova, Panchev, Pavlova, & Shtereva, 1994; Renard, Lemeunier, & Thibault, 1995; Renard, Voragen, Thibault, & Pilnik, 1990; Slavov et al., 2009; Vries, de Hansen, Søderberg, Glahn, & Pedersen, 1986).

In the present work, pectin was extracted from the apple pomace and was characterized with various physico-chemical

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parameters as well as XRD, ^{13}C NMR, FTIR and Raman spectroscopy. The extracted pectin from different sources as well as by different extraction processes was investigated as lipase (steapsin) inhibitor. Steapsin belongs to the class of digestive enzymes called lipases present in the pancreatic juice that catalyzes the hydrolysis of triglycerides (main constituent in vegetable oils/fat) to fatty acids and glycerol. To the best of our knowledge there is no published report on the lipase inhibition activity of pectin from apple waste.

2. Experimental

2.1. Materials

Two apple varieties of Royal (*Malus pumila*) and Golden (*Spondias dulcis*) from the Tikkar, Rohru, District Shimla (Himachal Pradesh, India) were used as the source of pectin. Steapsin (pancreatic lipase) (Sisco, India), hydrochloric acid, ethanol, sodium hydroxide, citric acid, magnesium sulphate (S.D. Fine Chem. Ltd., Mumbai, India), orlistat (Meyer Organics Pvt. Ltd., India), were used as received.

2.2. Pectin extraction

Apples were first washed and minced in an electric grinder. The crushed pulp was then pressed and the mash was dried, initially at room temperature and then at 50°C , to obtain a constant weight. The dry apple pomace pool was crushed and mixed, and the product was called apple flour. It was used as the raw material for all the assays made concerning pectin extraction.

Pectin was extracted under reflux in a condensation system at 97°C for 30 min (solute/solvent 1:50) using known weight of the apple flour (pool) as raw material and the dilute hydrochloric acid (pH 2.5) as the extractant. Similarly, pectin was extracted using dilute citric acid at pH 2.5. The de-ionized water was used to dilute both acids. Similar processes were repeated for the extraction of pectin from the flour obtained from the other variety of apple.

2.3. Isolation of pectin

Hot acid extract was pressed in a cheesecloth bag and the concentrated “juice” was cooled to 40°C . The apple pectin was precipitated by alcohol–juice treatment 2:1 (v/v). The precipitate was stirred for 10 min and then left undisturbed for 1 h in order to allow the pectin flotation. Following this procedure, the pectic substances remained at the surface of the alcohol/water mixture, and thus, it was easier to remove them in a quantitative way. The pressed pectin was dried to a constant weight at 55°C , cooled in a dessicator and the yield was calculated on a dry weight basis (initial weight of sample). The hardened pectin cake was broken up, grounded and sieved in order to obtain powdered pectin. To ensure complete removal of any acid or other impurities, the isolated pectin was dissolved in water and re-precipitated with distilled acetone. Different samples of the pectin were designated as $P_{\text{Mp-HCl}}$, $P_{\text{Mp-CA}}$, $P_{\text{Sd-HCl}}$ and $P_{\text{Sd-CA}}$, where P, Mp, HCl, CA and Sd stands for pectin, *Malus pumila*, hydrochloric acid, citric acid and *Spondias dulcis*, respectively.

2.4. Characterization of pectin powder

Pectin extracted from the two sources was characterized by FTIR (in KBr on Perkin-Elmer), Raman (HR MicroRaman Spectrometer, LabRAM HR 800) and ^{13}C NMR spectroscopy (in D_2O on superconducting FT-NMR spectrometer; 500 MHz, DRX-500 (Bruker)), and by investigating various physico-chemical parameters, i.e., equivalent weight, methoxyl content, alkalinity of ash, anhydrouronic acid, degree of esterification and acetyl value as reported elsewhere (Jain et al., 1984; Ranganna, 1986).

2.5. Steapsin inhibition study

0.1% by weight of steapsin was taken along with a commercial sample of mustard oil and dissolved in a buffer consisting of 13 mM Tris-HCl, 150 mM NaCl, and 1.3 mM CaCl_2 (pH = 8.0) (Nakai et al., 2005) were mixed in the reference set, and in the reaction set 0.1% $P_{\text{Mp-HCl}}$ (by weight of the oil) was taken in addition to the contents of the reference set. The reaction was carried out in the chemical reactor (AutoChem, USA) at 37°C under constant stirring for 4 h. The free acid generated in the hydrolysis reaction was estimated by micro-titration against 0.1 M NaOH using phenolphthalein as indicator (Chatterjee, Barbora, Cameotra, Mahanta, & Goswami, 2009). The same procedure was used with other pectin samples, and also with other substrates, including 2:1 ratio of pectin:steapsin. % inhibition (P_I) of lipase was calculated as:

$$P_I = \left[\frac{(V_2 - V_1)}{V_2} \right] \times 100, \quad (1)$$

where V_2 and V_1 are the volume of NaOH used in the reference set and reaction set, respectively. Effect of time on the lipase inhibition was studied with $P_{\text{Mp-HCl}}$ at the above conditions as function of time. All the tests were performed in duplicate.

3. Results and discussion

The yield and properties of pectin are dependent on the source and are also affected by the nature of the extraction process used. The use of citric acid as extractant yielded higher amount of pectin than hydrochloric acid (Table 1). Similar trends for the effect of hydrochloric acid and citric acid on the yield and properties of the extracted pectin are reported elsewhere (Virk & Soghi, 2004).

3.1. Characterization of pectin

FTIR spectra of different pectin samples have characteristic peaks at 3390.6, 2939.0, 1749.0 and 1052.1 cm^{-1} corresponding, respectively, to $-\text{OH}$, $-\text{CH}$, $\text{C}=\text{O}$ of ester and acid, and $-\text{COC}-$ stretching of the galactouronic acid (Fig. 1). FTIR spectra showed good match with the spectrum of the commercial pectin. There is an additional peak in the region $2361\text{--}2336\text{ cm}^{-1}$ of FTIR spectrum shows the presence of N-H stretching of some amidated salts. The low intensity of the Raman bands at 1164, 1184 and 1471 cm^{-1} proved that the all the extracted pectin samples were acetylated (Fig. 2). The region of $1200\text{--}1000\text{ cm}^{-1}$ contains skeletal

Table 1
Physico-chemical characteristics of pectin.

Pectin	Yield %	Physico-chemical parameters					
		Equivalent weight	% Methoxyl content	% Alkalinity of ash	% Anhydrouronic acid	% Degree of esterification	% Acetyl value
$P_{\text{Mp-HCl}}$	14.55	833.33	4.82	14.20	59.52	45.98	0.39
$P_{\text{Mp-CA}}$	16.65	1666.30	6.21	15.10	67.14	52.51	0.47
$P_{\text{Sd-HCl}}$	16.75	714.29	2.23	10.00	57.21	22.15	1.21
$P_{\text{Sd-CA}}$	18.79	909.09	5.68	18.00	64.32	50.14	0.34
Reported value	–	1030.92	5.30	–	70.50	42.68	0.50

The values shown in the bold are for the citric acid process.

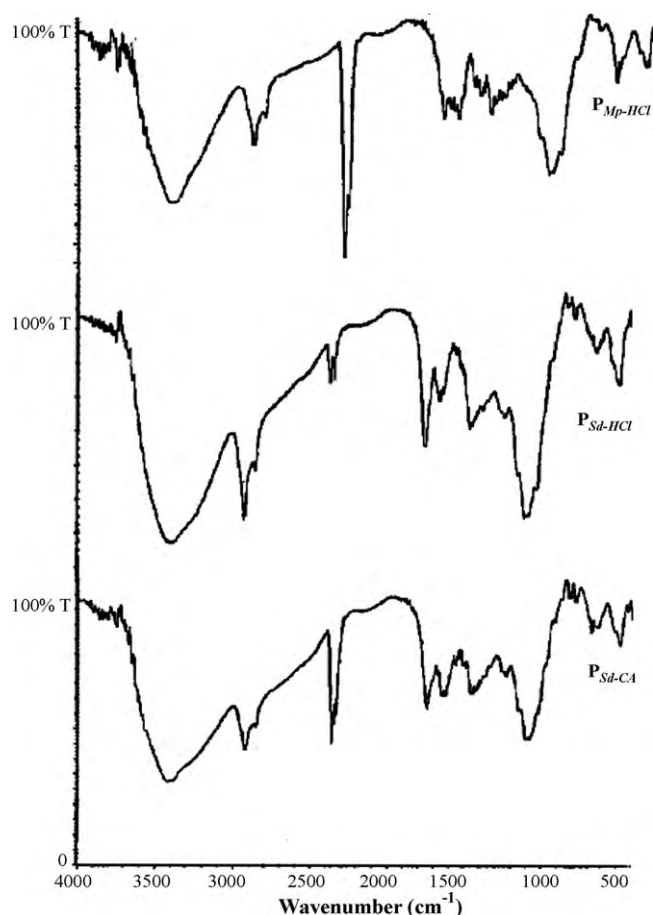


Fig. 1. FTIR spectra of pectin extracted from different sources.

C–O and C–C vibration bands of glycosidic bonds and pyranoid ring. An intense Raman bands at 410 cm^{-1} in the far-infrared region were also detected and assigned to the C–O–C torsion deformation (Synytsya, Copíková, Matejka, & Machovic, 2003). Effect of the extraction process and the nature of source are also evident from the differences in the intensity and position of the peaks in these spectra. ^{13}C NMR spectrum of $\text{P}_{\text{Mp-CA}}$ has signals at 102.13 and 76.39 ppm (Fig. 3). These were assigned to C-1 and C-5 of (1 $\rightarrow$ 5) linkage. The signals at 81.36, 69.64, 74.81 and 76.39 ppm were assigned to C-2, C-3, C-4 and C-5 of the galactouronic acid, respectively. The region 55.40–64.36 ppm was assigned to the signal originating from the 3-O-methylgalacturonic units (Hokputsa et al., 2004). ^{13}C NMR spectrum of $\text{P}_{\text{Sd-CA}}$ is similar to that described above with the sharp differences in the intensity of the main signals. X-ray diffraction study exhibited that pectin extracted from $\text{P}_{\text{Mp-HCl}}$ was more crystalline in nature as its XRD pattern has sharp and narrow diffraction peaks, while that of $\text{P}_{\text{Sd-HCl}}$ is less crystalline (Khodzhaeva et al., 2003) (Fig. 4).

3.2. Characterization of extracted pectin by physico-chemical parameters

The nature of the extraction process has pronounced effect on the equivalent weight of the extracted pectin. As compared to the value of 1030.9 for the equivalent weight of the commercial pectin, the equivalent weight of the pectin from $\text{P}_{\text{Mp-CA}}$ was found to be the highest (1666.67), while the lowest equivalent weight (833.33) was observed for $\text{P}_{\text{Mp-HCl}}$ (Table 1). It is suggested that pectin was degraded in the extraction medium that had hydrochloric acid. Some of the characteristics of the extracted pectin ($\text{P}_{\text{Sd-HCl}}$) like

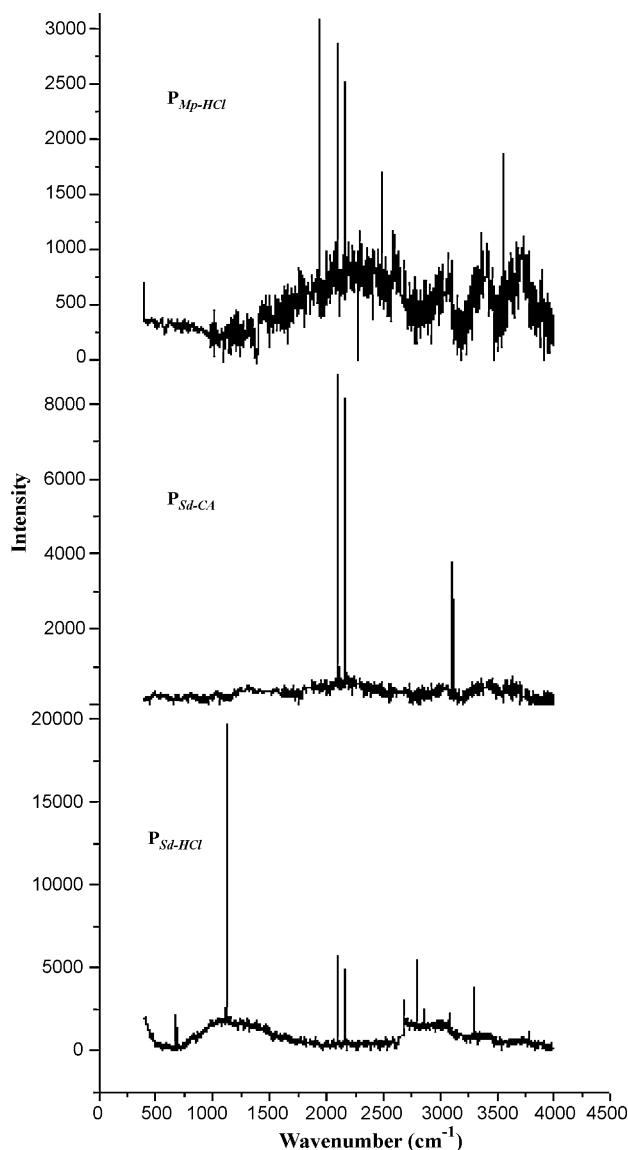


Fig. 2. FT-Raman spectra of pectin extracted from different sources.

methoxyl content (2.23%), anhydrouronic acid content (57.21%), degree of esterification (22.15%), and the acetyl values (1.21%) as compared to 7.4, 74.1, 72.4 and 0.21% those reported in the literature for the pectin extracted from the *Spondias dulcis* pomace (Jain et al., 1984; Sharma, Lal, Kumar, & Goswami, 1985). From the anhydrouronic acid contents and acetyl values obtained for these four pectin samples it is implied that these have low tendency to form gels.

3.3. Steapsin inhibition studies

P_i of steapsin by the extracted pectin is presented in Fig. 5. The reaction was carried out in the presence of the Ca^{2+} ions to enhance the catalytic activity of lipase. Ca^{2+} ions form the insoluble Ca-salts of fatty acids released in the hydrolysis of oils, thus avoiding the product inhibition (Sharma, Bardhan, & Patel, 2009), while Ca^{2+} itself has no effect on the enzyme activity (Posner & Morales, 1972). A series of experiments were carried to study the interaction of lipase/pectin or pectin/oil. The efficacy of the pectin as lipase inhibitor is very high even when used in 1:1 steapsin and pectin ratio. The P_i did not change much when the pectin:lipase ratio of 2:1 was used. There was a lack of sharp substrate selectivity

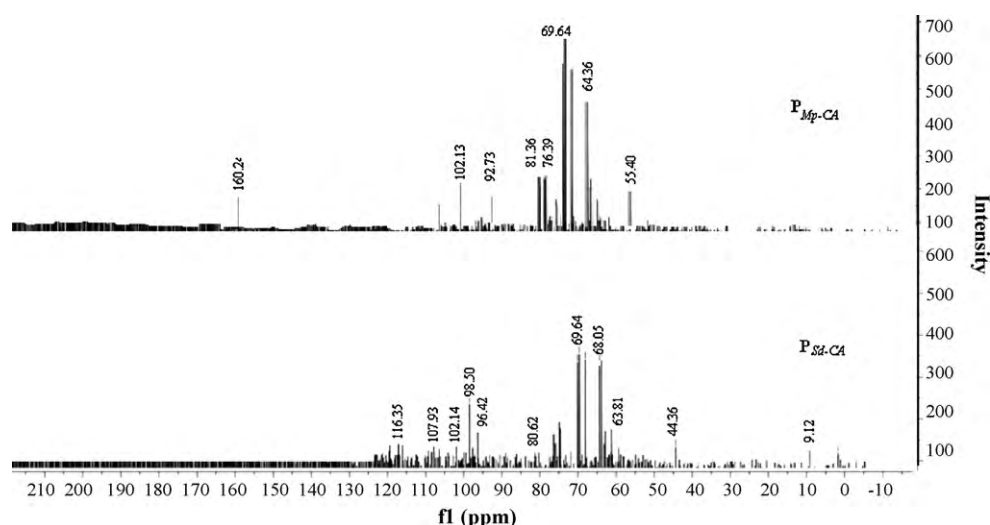


Fig. 3. ^{13}NMR spectra of pectin extracted from different sources.

implying that the formation of the pectin and lipase complex does not permit interaction of lipase with the substrate, i.e., oil. Tetrahydrolipstatin (orlistat) was used as the positive control to compare its steapsin inhibitory action with pectin (Fig. 5). Tetrahydrolipstatin exhibited the maximum P_i (97.44%) in the sunflower oil (tetrahydrolipstatin:steapsin, 1:1), which is higher than that observed with pectin using the same pectin:steapsin ratio. From the preliminary investigation, it was observed that P_{Mp-CA} exhibited the highest lipase inhibition; hence it was selected for the further studies to study steapsin inhibition as function of time, again using

tetrahydrolipstatin as the positive control. In both the cases, the inhibitory action was observed to be fast, as appreciable inhibition was observed even within 30 min, and it increased with time (Fig. 6).

The scheme of the steapsin inhibition is shown in Fig. 7. The mechanism of the steapsin inhibition is by the competitive inhibition. The lipase that was interacted with different pectin samples at pH 8.0 for 30 min was found to be inactive in the presence of oil/substrate as pectin forms complex with the steapsin. The biopolymers are reported to form complexes with lipase. The formation of the lipase–chitosan complex and consequent lipase inhibition has been reported in the literature (Ostanina, Varlamov, & Iakovlev, 2008). The formation of a lipase–pectin complex in the presence of substrate becomes competitive and results in the lipase inhibition. It is argued that in the present study, the low equivalent weight and low gelling tendency of these pectin samples are two key factors those affect the steapsin–pectin complex formation. Pectin in the dilute solution dissociates like organic acids. This attribute of pectin makes it a suitable candidate for the pancreatic lipase inhibition, as pectin being a weak acid, it does not dissociate in the low pH gastric juice. It is reported in the literature that pectin binds covalently to the active site of the pancreatic lipase

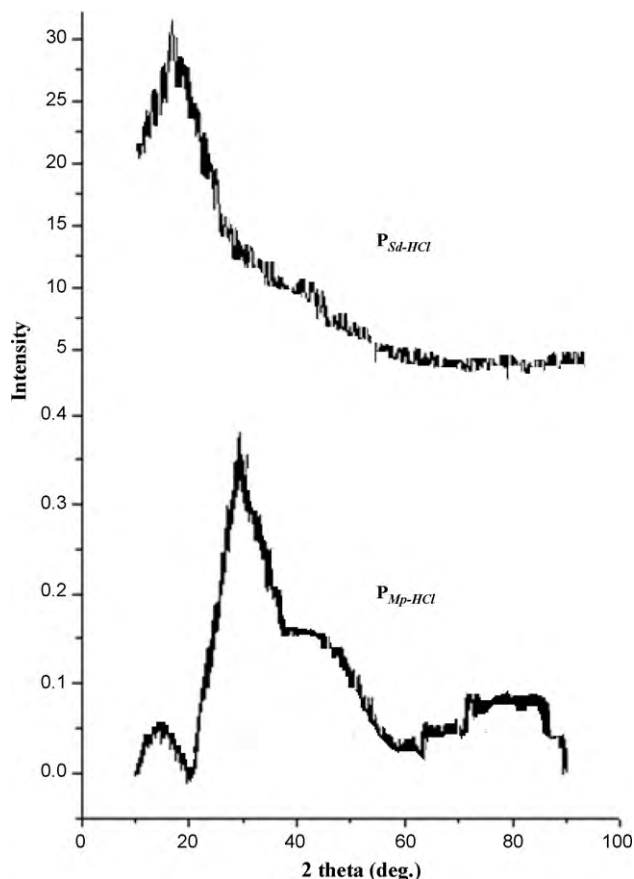


Fig. 4. XRD of pectin extracted from different sources.

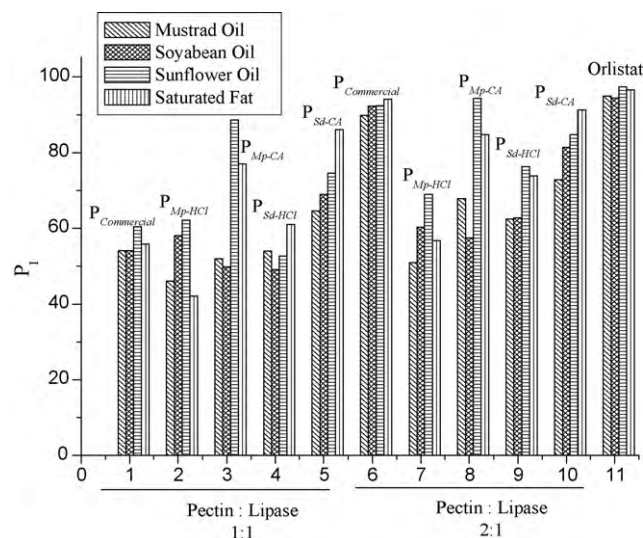


Fig. 5. Percent inhibition (P_i) of steapsin by the extracted pectin.

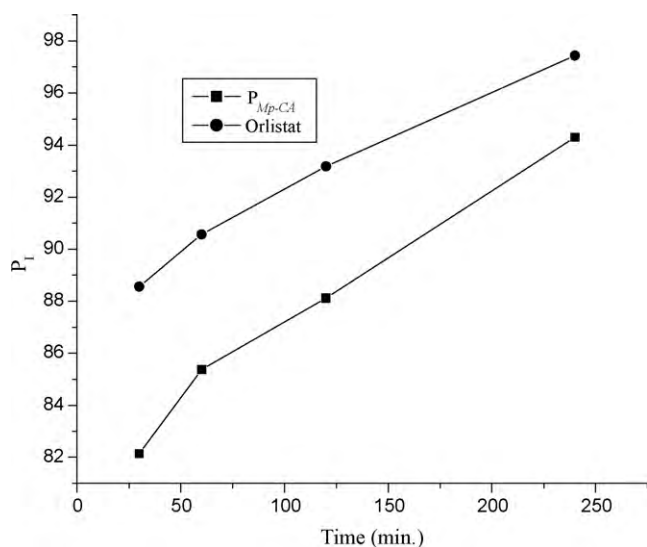


Fig. 6. Percent inhibition (P_i) as a function of time at 37 °C and pH 8.0 with P_{Mp-CA} in sunflower oil.

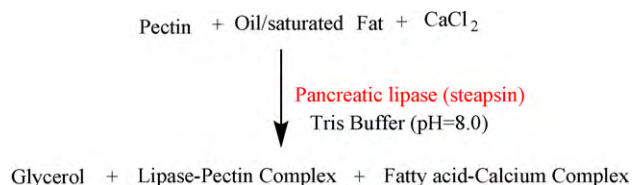


Fig. 7. Scheme of lipase (steapsin) inhibition.

and forms a stable complex (Cudrey et al., 1993). It is suggested that the $-CO_2H$ groups of pectin protonates histidine and may be even the hydroxyl group of serine of the active serine–histidine–aspartic acid/glutamic acid triad of lipase, and that stops the initiation of the relay mechanism necessary for the initiation of mechanism of lipase action. Further, contribution to the pectin–lipase interaction also comes in the form of intermolecular hydrophobic interactions from the high methyl contents of pectin (Oakenfull, 1991). The later also contributes in the formation of complex with lipase.

4. Conclusions

Pectin was extracted from two different sources (apple pomace). The extraction of pectin with citric acid was found to be more efficient and less polymer chain disruptive process. The physico-chemical parameters of the extracted pectin are dependent both on the nature of source as well as extraction process. All the four samples of the extracted pectin are potent lipase (steapsin) inhibitors, and the pectin extracted from the *Malus pumila* using the aqueous citric acid as extractant exhibited the highest lipase inhibition. The efficacy of the pectin as lipase inhibitor is appreciable, yet lower than that of the commercial lipase inhibitor, tetrahydrolipstatin. The mechanism of inhibition is proposed as competitive since pectin forms complex with steapsin. The extracted pectin has potential for use in the anti-obesity formulations and personal care products.

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