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# Effect of Enzyme Concentration on Quality of Apple Pomace Pectin

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**A B S T R A C T**

Pectin yield is usually depends upon the extraction conditions such as temperature, extraction time, pH, drying method and most importantly type of extraction solvents. During present study, the enzymatic extraction of pectin from apple pomace has been shown to achieve higher recovery of pectin and is regarded as an environmentally safe technique. Different ecofriendly enzymes viz., cellulase, hemicellulase, amylase and xylanase were added at different concentrations to rehydrated apple pomace powder for different time. Among different concentration and time of enzyme reaction, cellulase (12µg for 2 hr) was found better with highest pectin yield of 20.42 per cent for apple pomace pectin extraction. The extracted pectin contains high equivalent weight 1710.32, methoxyl content 5.94 per cent, with highest anhydrogalacturonic acid 44.01 percent and a jelly grade of 80. Thus, the pectin extracted by using cellulase enzyme was found to be the best among other enzymes used for extraction of pectin on the basis of pectin yield and its quality parameters.

## Introduction

Apple pomace is obtained as a main by-product of juice industry, consisting of 25-35 percent of dry mass of apples (Kaushal *et al.*, 2002). It is the solid residue consisting of peel, core, seed, calyx, stem and soft tissue (Francielo *et al.*, 2008). Apple pomace is a rich source of pectin (Bhushan *et al.*, 2008) containing 16.95% pectin with a very high biological oxygen demand i.e. 240 -19000 mg/l (Joshi and Joshi, 1990). Several methods have been tried to extract pectin involving acids, alkalines and enzymes. Pectin's are obtained from apple pomace and citrus peels in a chemical way industrially with strong acids such as oxalic (Koubala *et al.*, 2008), hydrochloric (Hwang *et al.*, 1998), nitric (Constenla and Lozano, 2003) and sulphuric acids (Garna *et al.*, 2007) which are regarded as conventional acid extraction methods (Yapo, 2009). Although these chemical procedures have advantages from an efficient and economical point of view, but they may cause environmental problems by producing hazardous contaminants that need cumbersome methods for treatment. Serious consumer concerns about chemical additives have become so dominant that the growth rate of natural products in the food industry has begun to rise (Sloan, 2010). Therefore, some efforts are required to minimize the use of harmful chemicals and replace with enzymes for pectin isolation.

Enzymatic extraction has been conducted with polygalacturonase (Contreras-Esquivel *et al.*, 2006), hemicellulase and cellulase (Shkodina *et al.*, 1998), protease (Zykwinska *et al.*, 2008) and microbial mixed enzymes (Ptichkina *et al.*, 2008). The enzymatic extraction has been shown to achieve higher recovery of pectin than other extraction methods (Panouille *et al.*, 2006; Ptitchkina *et al.*, 2008). Moreover, enzymatic extraction is regarded as an environmentally safe technique as enzymes degrade the pectin by selective depolymerisation. However, Panouille *et al.*, (2006) found enzymatic extraction more expensive than other extraction methods, such as acid extraction. Yuliarti *et al.*, (2011) evaluated the effect of celluclast 1.5L on the physicochemical characterization of gold kiwifruit pectin and with different enzyme concentration (0.1 ml/kg, 1.05 ml/kg and 2.0 ml/kg). Panouille *et al.,* (2006) reported that enzymes can extract pectin with a higher yield from a smaller mass as compared to acid extractant. Thus, the present investigations were carried out to optimize ecofriendly enzyme and process for extraction of high grade pectin from apple pomace.

## Materials and Methods

Fresh apple pomace procured from Himachal Pradesh Horticultural Produce Marketing and Processing Corporation Ltd (hpmc) Fruit Processing Plant, Parwanoo, Distt. Solan (H.P.) dried in the mechanical dehydrator (55±2$^{o}$C) to a moisture content of 9.28 percent. Fresh and dried apple pomace was analyzed for different quality characteristics. Dried apple pomace was turned into powder by passing through the Pulverizer (6000-18000 rpm) ultra-centrifugal mill by Bajaj process pack, Noida, UP.

### Optimization of enzymes for pectin extraction

Rehydrated apple pomace powder was treated with different enzymes viz., cellulase, hemicellulase, amylase and xylanase procured from Sigma Aldrich.

The enzymatic activity of cellulase from *Trichoderma viride* off white powder was 0.8 -1.8U/mg, hemicullase from *Aspergillus niger* in powder form with 0.3-3.0 unit/mg, xylanase from *Thermomyces lanuginosus* with activity of 2500units/g and maltogenic amylase A2986-10G. To enhance the activity of enzyme the pH of rehydrated pomace was regulated at 4.5 (citrate buffer). Keeping pectin yield as an indicator, specific concentration and time of extraction of enzyme were optimized.

### Physico-chemical analysis of apple pomace and pectin

Moisture, total soluble solids, titratable acidity, ascorbic acid, ash, crude protein, pectin as calcium pectate, equivalent weight, methoxyl content, anhydrogalacturonic acid content and degree of esterification was determined using methods detailed by Ranganna, (1997). Reducing and total sugars were estimated as per the method of Lane and Eynon, (1923) as described by Ranganna. Crude fibre was calculated by method given by Gould, (1978).

### Statistical analysis

Data pertaining to physico-chemical parameters of dried apple pomace, pectin's extracted from the experiments like extraction methods viz., use of chemicals and enzymes and different precipitating agents were analyzed by Completely Randomized Design (Cochran and Cox, 1967). Three replications were recorded for each characteristic.

## Results and Discussion

### Physico-chemical characteristics of apple pomace

The fresh as well as dried apple pomace was analyzed for different quality attributes. The results for dried pomace shows that the moisture content reduced to 9.28 from 81.35 percent with increased total soluble solids, total sugars, reducing sugars, titratable acidity, ascorbic acid and pectin contents 44.40oB, 38.05%, 14.30%, 1.81%, 13.07mg/100g and 14.66% respectively as compared to fresh pomace 8.65oB, 6.25%, 2.17%, 0.27%, 3.40mg/100g and 2.33% respectively (Table 1).

The values were found within the range reported by Devarajan (1997) and Kaushal (2008). Joshi and Attri (2006) also reported similar results with higher total sugar content (48.00 percent) and lower total ash content (1.82 per cent) on dry weight basis.

### Effect of enzyme concentration and treatment time on the pectin yield

Different ecofriendly enzymes viz., cellulase, hemicellulase, amylase and xylanase were added at different concentrations to rehydrated apple pomace powder for different time intervals (1 to 5 hours) to study the effect of enzyme concentration and time interval on pectin yield from apple pomace powder at temperature of 50$^{o}$C and pH 3.5. The best combination of concentration and time on the basis of pectin yield was selected and the effect on quality of pectin extracted was studied.

Data in Table 2 show that in case of cellulase enzyme 2 h treatment time and 12 µg/g concentration found to give the highest pectin yield of 20.42 per cent from apple pomace powder. Further, among different concentration of hemicellulase enzyme higher yield was recorded with 12 µg/g concentration treatment for 3 hours observed with highest pectin yield of 19.87 percent.

The perusal of data in Table 2 shows the effect of amylase enzyme concentration and 3 h treatment time and 20 µg/g concentration of amylase enzyme was found to give the highest yield of 17.95 per cent pectin. Among the different concentration of xylanase enzyme mean higher yield was recorded with 12 µg/g enzyme concentration with 4 h enzyme treatment time results in the highest yield of pectin (19.14 %).

**Table.1** Physico-chemical composition of apple pomace

| Attribute(s) | Fresh Pomace | Dried Pomace |
|---|---|---|
| **Moisture, %** | 81.35±4.05 | 9.28±1.12 |
| **TSS, °Brix** | 8.65±0.51 | 44.40±5.74 |
| **Titratable acidity, % as malic acid** | 0.27±0.05 | 1.81±0.34 |
| **Total sugars, %** | 6.25±0.79 | 38.05±7.68 |
| **Reducing sugar, %** | 2.17±0.16 | 14.30±0.75 |
| **Ascorbic acid, mg/100g** | 3.40±0.65 | 13.07±1.63 |
| **Crude fibre, %** | 5.41±0.73 | 31.31±4.83 |
| **Crude protein, %** | 2.70±0.70 | 6.12±1.89 |
| **Ash content, %** | 0.85±0.14 | 5.44±0.38 |
| **Pectin, % as calcium pectate** | 2.33±0.60 | 14.66±3.83 |

**Table.2** Effect of enzyme concentration and time interval on pectin yield

| Time intervals (I) / Enzyme Conc. (µg/g) | Pectin yield (%) | | | | | | CD (0.05) |
|---|---|---|---|---|---|---|---|
| | **1 hr** | **2 hr** | **3 hr** | **4 hr** | **5 hr** | **Mean** | |
| | **Cellulase enzyme** | | | | | | |
| **4** | 11.58 | 14.46 | 17.26 | 18.21 | 18.02 | **15.91** | C= 0.16 |
| **12** | 15.55 | 20.42 | 18.76 | 14.96 | 13.08 | **16.55** | I= 0.21 |
| **20** | 15.61 | 16.40 | 14.32 | 12.01 | 10.94 | **13.86** | C×I= 0.36 |
| **Mean** | **14.25** | **17.09** | **16.78** | **15.06** | **14.01** | | |
| | **Hemicellulase enzyme** | | | | | | |
| **4** | 10.98 | 11.82 | 13.12 | 14.43 | 14.13 | **12.90** | C= 0.23 |
| **12** | 13.43 | 16.36 | 19.87 | 18.18 | 16.94 | **16.96** | I= 0.30 |
| **20** | 14.03 | 17.44 | 16.96 | 15.88 | 14.62 | **15.79** | C×I= 0.51 |
| **Mean** | **12.81** | **15.21** | **16.65** | **16.16** | **15.23** | | |
| | **Amylase enzyme** | | | | | | |
| **4** | 10.13 | 10.50 | 11.45 | 12.25 | 11.90 | **11.25** | C= 0.18 |
| **12** | 10.90 | 12.20 | 13.73 | 15.62 | 14.36 | **13.36** | I= 0.24 |
| **20** | 12.73 | 15.56 | 17.95 | 16.25 | 15.70 | **15.64** | C×I= 0.41 |
| **Mean** | **11.25** | **12.75** | **14.38** | **14.71** | **13.99** | | |
| | **Xylanase enzyme** | | | | | | |
| **4** | 11.22 | 13.42 | 14.98 | 16.18 | 15.12 | **14.18** | C= 0.18 |
| **12** | 12.12 | 14.68 | 16.27 | 19.14 | 18.41 | **16.12** | I= 0.23 |
| **20** | 12.82 | 15.34 | 17.48 | 15.43 | 11.36 | **14.49** | C×I= 0.41 |
| **Mean** | **12.05** | **14.48** | **16.24** | **16.92** | **14.96** | | |

**Table.3** Characteristics of pectin extracted by ecofriendly enzymes with optimized concentration and treatment time

| Enzyme Extractants / Quality Characteristics | Without Enzyme | Cellulase | Hemicellulase | Amylase | Xylanase | Mean | $CD_{0.05}$ |
|---|---|---|---|---|---|---|---|
| **Pectin yield (%)** | 17.12 | 20.42 | 19.87 | 17.95 | 19.14 | 18.90 | 0.47 |
| **Ash (%)** | 2.05 | 3.01 | 2.98 | 2.87 | 2.93 | 2.77 | 0.23 |
| **Equivalent weight** | 1041.66 | 1710.32 | 1579.76 | 1418.67 | 1566.23 | 1463.33 | 47.06 |
| **Methoxyl Content (%)** | 5.15 | 5.94 | 5.61 | 5.18 | 5.53 | 5.48 | 0.21 |
| **Anhydroglacturonic acid (%)** | 46.13 | 44.01 | 42.98 | 41.82 | 42.63 | 43.51 | 1.01 |
| **Degree of esterification (%)** | 63.38 | 76.62 | 74.09 | 70.33 | 73.64 | 71.61 | 0.87 |
| **Jelly Grade** | 70.00 | 80.00 | 76.00 | 65.00 | 74.00 | 49.00 | 9.21 |

**Table.4** Quality characteristics of enzyme extracted pectin

| Type of pectin / Quality Characteristics | Cellulase enzyme (12 µg/g) | Commercial Pectin |
|---|---|---|
| **Pectin yield (%)** | 20.48 | - |
| **Ash (%)** | 3.05 | 1.04 |
| **Equivalent weight** | 1732.55 | 892.56 |
| **Methoxyl Content (%)** | 6.01 | 8.01 |
| **Anhydroglacturonic acid (%)** | 44.28 | 65.19 |
| **Degree of esterification (%)** | 77.06 | 69.75 |
| **Jelly Grade** | 84.00 | 125 |

## Optimization of enzymes for extraction of pectin

Enzyme concentration of 12 µg/g was found best for cellulase (2 hours), hemicellulase (3 hours) and xylanase (4 hours) with pectin yield of 20.42, 19.87 and 19.14 percent respectively (Table 2). Whereas, in case of amylase 20 µg/g enzyme concentration (3 hours) was found best with 17.95 percent pectin. According to Feng *et al.,* (2011) at high concentration and long extraction time leads to reduced pectin yield, because extending the extraction time induces degradation of pectin molecular chains under the action of enzymes, eventually affecting pectin yield.

The results presented in Table 3 shows that the yield of 17.12 percent pectin recovered without enzyme. The yield by using enzymes was low as compared to pectin extraction with citric acid (26.88%). Similar results were reported by Lim *et al.,* (2012), Min *et al.,* (2010) during pectin extraction from apple pomace. Panouille *et al.,* (2006) and Yuliarti *et al.,* (2011) reported that enzymes can extract pectin with a high yield. On basis of pectin yield, cellulase enzyme was found better and thus optimized for pectin extraction. The quality characteristics of extracted pectin show that the highest jelly grade was obtained by using cellulase enzyme as compared to lowest jelly grade by amylase. The pectin extracted by cellulase enzyme found of better quality with equivalent weight 1710.32, anhydroglacturonic acid 44.01%, methoxyl content 5.94%, degree of esterification 76.62% and ash content of 5.94 percent (Table 3). According to Ramli and Asmawati, (2011) the increase or decrease of equivalent weight might be dependent on the free galacturonic acids. The anhydrogalacturonic acid content of pectin extracted by using enzymes was low as compared to chemically extracted pectin this might be due to the solubilization of non-pectic polysaccharides compounds during pectin extraction with enzymes, which are negatively contributing to the purity of galacturonic acid. The results were in accordance to Lim *et al.*, (2012); Mohd *et al.*, (2012). Min *et al.*, (2010) reported higher degree of esterification for pectin's extracted by physical/enzymatic treatments than chemically extracted pectin.

## Quality characteristics of apple pomace pectin

Table 4 shows the data of quality characteristics of enzyme extracted apple pomace pectin and compared with commercial pectin. Data show that the apple pomace pectin extracted by cellulase enzyme was found better as compared to the commercial pectin.

The study concludes that the apple pomace can be utilized for the extraction of pectin by using enzymatic (Cellulase @ 12µg/g) methods followed by precipitation with ethanol and drying to 8 percent moisture under dehumidifier conditions with yield of 20.48 percent pectin. Further, on the basis of quality parameters, the apple pomace pectin extracted by cellulase enzyme was found of best quality as comparable with commercial pectin. Therefore, the apple pomace can be a successful substrate and cellulase can be a better extractant for isolation and production of pectin at pilot scale.

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