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Optimized Extraction and Characterization of Pectin from Gooseberry and Strawberry Pomace Validated by Response Surface Methodology

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Abstract: The potential of gooseberry and strawberry fruit pomace as a source of pectin was evaluated. Highest pectin yield i.e., 19.68% and 1.667% was obtained using HNO3 as compared to citric acid and HCl at 90°C from gooseberry and strawberry pomace respectively. Extracted pectin was further characterized as high ester pectin and can be used as alternative source of pectin in food industry. A central composite design was employed to validate the optimization of pectin extraction with HNO3. The expected values obtained by Response surface methodology validate the results of optimization of pectin extraction.

Key words: Gooseberry · Strawberry · Pomace · Pectin · Degree of Esterification · Response surface methodology · Central composite design

INTRODUCTION

Pectin is derived from Greek word that means "congealed and curdled". It is an abundant ubiquitous and multifunctional structural heteropolysaccharide mainly present in primary cell wall of terrestrial plants. Pectin present in middle lamella between plant cells helps to bind the cells together [1]. During fruit ripening enzymes pectinase and pectin esterase hydrolyse pectin resulting in fruit softening. Pectin has a complex polysaccharide with α-(1→4)-linked D-galacturonic acid backbone. Preparation consist of substructural entities that depend on their source and extraction methodology. Commercial extraction of pectin is also accompanied by extensive degradation of neutral sugar-containing side chains. Majority of structure consists of homopolymeric partially 6-methylated and 2- and 3- acetylated poly-α-(1→4)-D-galacturonic acid residues ('smooth') but there are substantial 'hairy' non-gelling areas of alternating α -(1→2)-L-rhamnosyl-α -(1→4)-D-galacturonosyl sections containing branch-points with mostly neutral side chains (1 - 20 residues) of mainly α -L-arabinofuranose and α -D-galactopyranose (rhamnogalacturonan I). Pectin from some sources comprised of xylogalacturonan blocks of α-(1→4)-D-galacturonic acid units, partially substituted at O-3 position with single non-reducing β-D-xylopyranose with longer (dimer to octamer) β-D-xylopyranose chains. Pectin is a polymer of α- galacturonic acid with a variable number of methyl ester groups [2]. Some of the carboxylic groups of galacturonic acid in pectin chains are methyl esterified and the percentage of esterified groups is expressed as DE (degree of esterification). Depending upon DE, pectin is divided into two majors groups: high–ester pectin, with DE higher than 50% and low-ester pectin with DE lower than 50% [3]. It is also used as functional food ingredient as a gelling agent, thickening agent and stabilizer [4]. Commercial pectins are mainly derived from citrus peel or apple pomace or both and also from by-products of juice industry. Apple pomace and citrus peel contain 10-15% and 20-30% of pectin respectively on dry matter basis. Pectin is also obtained from sugarbeet waste of sugar factory, sunflower heads (seeds for edible oil) and mango waste [5]. Pectin is available as a solution, extract and blended power. Pectin is also being studied as stabilizer for acidic protein drinks, such as drinking yoghurt and jam making. It is being used as a fat substitute in baked goods spreads, ice-creams and salad dressings. In medicine, pectin is used against constipation and diarrhoea, in throat lozenges as a demulcent, in wound healing, in medical adhesives such as colostomy devices and natural prophylactic against poisoning. It is effective in removing lead and mercury from gastrointestinal tract and respiratory organs. When injected intravenously, pectin shortens blood coagulation time and thus used in controlling hemorrhage or local bleeding [6]. In pharmaceuticals pectin is used as a carrier of a variety of drugs for their controlled release. It is used in pharmaceutical preparation as filler, as an

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agglutinator in blood therapy and also to glaze candied fruits. Lemons, oranges and grapefruits are rich in pectin and may help in reduced rate of tumor formation [7]. Pectin confer health benefit by lowering blood cholesterol and low-density lipoprotein [2]. In recent years, people have become aware about their health. There is increase production of processed fruits products and juices. This is also accompanied with enormous increase in fruit waste which poses disposal problem. This waste can be effectively used to manufacture useful byproducts. Pectin is one such valuable byproduct that can be obtained from fruit wastes. The suitability of pectin for different purpose is determined by their characters viz., anhydrouronic acid content, methoxyl content, degree of esterification and acetyl value [7].

The gooseberry (*Ribes uva-crispa* and syn. *Ribes grossularia*) is an edible fruit of species of *Ribes* that is native to Europe, Northwestern, West, South and Southeast Asia. *Emblica officianalis* an Indian variety of gooseberry.

Strawberry (*Fragaria X ananassa)* belongs to family *Rosaceae* and is widely cultivated as an important commercial crop in many temperate regions all over the world. Botanically, the plant is a runner (creeper).

Gooseberries confer health benefit and well known for its nutritional qualities being rich in antioxidants, polyphenols, tannins, micronutrients and folate. They are used in various value added products like preserve (murabba), candy, juice, pickle and powder [8]. Phenolics of strawberries are best known for their antioxidant and anti-inflammatory action and possess antimicrobial, anti-allergy and antihypertensive properties [9]. Thus these two fruits were selected to extract the maximum pectin by developing a approach with better understanding of the relationship between different variables viz., temperature, pH and extraction time. The present communication envisages the optimized pectin extraction from these fruits.

MATERIALS AND METHODS

Plant Material: *Ribes uva-crispa* (Gooseberry) and *Fragaria X ananassa* (Strawberry) were used for the study.

Chemicals: All the chemicals used in study were of analytical grade. Petroleum ether, ethanol, nitric acid, sulphuric acid, anthrone, phenol red indicator (RANKEM, HIMEDIA).

Preparation of Pomace: Fruits were washed and pressed, remaining pomace was dried in a air-circulate oven at 55°C till the weight was constant. Dried pomace was ground with pestle mortar and used as raw material for pectin extraction and characterization assays. Dried powder was packed in polyethylene bag and stored at - 20°C.

Chemical Analysis: Dried pomace was analyzed for moisture, lipid, total protein and sugar contents.

Moisture Content: Fruit pomace was weighed and incubated in oven for 24 h at 55°C. The sample was again weighed after incubation. Moisture content was calculated according to Association of Official Analytical Chemist (AOAC) [10].

Moisture content = weight of sample before incubation – weight of sample after incubation.

Total Fat Content: A thimble of filter paper was prepared and 3 g of sample powder was placed in it and extracted with 250 ml of petroleum ether using soxhelt apparatus until 3-4 cycles of evaporation were completed. Then thimble containing residue was weighed to determine fats. Fat content was calculated using the following formula [10].

Fat content = [(weight of thimble containing sample powder before evaporation – weight of thimble containing sample residue after evaporation) – weight of thimble paper]

Total Sugar Content: Total sugar content was determined by Anthrone method using standard curve of glucose (100µg/ml) [11].

Total Protein Content: Total protein content was determined by Biuret method using standard curve of BSA (5mg/ml) [12].

Pectin Extraction from Fruit Pomace

Experimental Design and Statistical Analysis: RSM (Response Surface Methodology) was used to analyze and validate the conditions for pectin extraction from fruit pomace. The central composite design with three variables viz., temperature, pH and extraction time (Et) was employed. The minimum and maximum values were set for all three variables. RSM provided 20 runs for pectin extraction. All the experiments carried out orderly

Table 1: Experimental and coded levels of three variables employed for pectin extraction in the central composite design experimental design

	Level		
Variables	Low level (-1)	Level (0)	High level (+1)
Temperature	40	65	90
pH	1.20	1.90	2.60
Extraction time	10.00	50	90.00

and analyzed the best condition for pectin extraction. The analysis of variance (ANOVA) was applied to validate the model. Table 1 shows the experimental and coded levels of three variables employed for pectin extraction in the central composite design.

Pectin Extraction: Pectin was extracted by the method of Kliemann *et al*. [13]. Under the standardized conditions with respect to extraction time, pH and temperature. Dry mass (5 g) was subjected to extraction by adding 250 ml water. The pH was adjusted to 2.5 with 0.5 M HCl, 0.5 M HNO3 and 0.5 M citric acid. The mixture was heated to 90°C and extraction was carried out with continuous stirring for 90 min. The hot acid extract was filtered through the muslin cloth and filtrate was cooled to 4°C. The filtrate was coagulated with equal volume of 96% ethanol and left for 1 h. The coagulated pectin was separated by filtration, washed with 70% acidic ethanol (0.5% HCl), then with 70% ethanol to a neutral pH and finally with 96% ethanol. The resulting material was dried overnight at 55°C in hot air oven. The pectin yield was calculated using following equation:

*y*pec (%) = 100 (P/Bi)

where ypec is extracted pectin yield in per cent (%), P is the amount of extracted pectin in grams and Bi is the initial amount of sample powder (5 g) [14].

Characterization of Pectin

Equivalent Weight: It was determined by titration with sodium hydroxide to pH 7.5 using phenol indicator. Pectin (0.5g) was taken in 250-ml conical flask and diluted with 5ml ethanol. Sodium chloride (1g) was added to sharpen the end point. 100ml of deionised water and six drops of phenol red indicator were added. Solubility of all pectin substances was ensured. This was slowly titrated with 0.1N NaOH until the colour of indicator changes (pH: 7.5) {Titration A}. The neutralized solution was used for determination of methoxyl content [14].

Equivalent Weight = (weight of sample×1000)/(ml of alkali×normality of alkali)

Methoxyl Content: The methoxyl (MeO) content was determined by adding 25 ml of 0.25 N NaOH to the neutral solution, mixed thoroughly and allowed to stand for 30 min at room temperature in a stoppard flask. Then 25 ml of 0.25N HCl was added and titrated against 0.1N NaOH to the end point (i.e. appearance of pink colour) {Titration B}. The methoxyl content was calculated using following equation:

MeO % = [meq titration B×31×100] / [weight of sample (mg)]

31 is the molecular weight of methoxyl.

Anhydrouronic Acid (AUA): Anhydrouronic acid content is essential to determine purity and degree of esterification and evaluation physical properties. AUA was calculated as follows (Nazaruddin *et al*. 2003).

%AUA = (176×100) / z

where 176 is the molecular weight of AUA and

Z = [weight of sample (mg)] / [meq titration A + meq titration B]

Degree of Esterification: Degree of esterification (DE) of pectin was calculated as follows [15]:

%DE = (176×Meo×100) / (31×AUA%)

where MeO is the % methoxyl content.

RESULTS AND DISCUSSSION

Chemical analysis of gooseberries and strawberries fruit pomace was done and their moisture, fat, sugar and protein content were determined. The results are summarized in Table 2. The fruit pomace of gooseberries contained 64.7% moisture, 0.67 g fat, 62±0.372 µg/ml sugar and 3.15±0.605 mg/ml protein while strawberries pomace contained 259.793% moisture, 0.215g fat, 61± 0.372 µg/ml sugar and 0.90±0.796 mg/ml protein. The Strawberry pomace was rich in moisture content as compared to gooseberry whereas gooseberry had relatively higher fat and protein content. Sugar content of both fruits was comparable.

Table 2: Characterization of fruit pomace

	Analysis of Gooseberry and Strawberry Pomace	
	Gooseberry	Strawberry
Moisture Content	64.7%	25.9%
Fat Content	0.67 g	0.215g
Sugar Content	62±0.372µg/ml	61±0.372µg/ml
Protein Content	3.15±0.605mg/ml	0.90±0.769mg/ml

The values are mean ± SD of three different experiments

Table 3: Variables, Levels and Response of pectin yield (Gooseberry) with respect to temperature, pH and extraction time

Std	Run	Block	Factor A: Temperature (°C)	Factor B: pH	Factor C: Extraction time (min)	Response: Pectin yield (%)
11	1	1	65	0.72	50	18.55
1	2	1	40	1.20	10	19.66
8	3	1	90	2.60	90	19.68
12	4	1	65	3.08	50	17.5
4	5	1	90	2.60	10	20.55
5	6	1	40	1.20	90	19.23
7	7	1	40	2.60	90	19.68
2	8	1	90	1.20	10	22.45
15	9	1	65	1.90	50	21.69
13	10	1	65	1.90	-17.27	20
3	11	1	40	2.60	10	21.72
10	12	1	107.04	1.90	50	24.56
18	13	1	65	1.90	50	21.96
19	14	1	65	1.90	50	21.99
17	15	1	65	1.90	50	22
9	16	1	22	1.90	50	18.14
16	17	1	65	1.90	50	23
14	18	1	65	1.90	117.27	21.56
20	19	1	65	1.90	50	23.69
6	20	1	90	1.20	90	25.01

Table 4: Variables, Levels and Response of pectin yield (Strawberry) with respect to temperature, pH and extraction time

Std	Run	Block	Factor A: Temperature (°C)	Factor B: pH	Factor C: Extraction Time (min)	Response: Pectin Yield (%)
15	1	1	65	1.90	50	1.22
5	2	1	40	1.20	90	1.31
12	3	1	65	3.80	50	0.85
17	4	1	65	1.90	50	1.23
3	5	1	40	2.60	10	0.56
14	6	1	65	1.90	117.17	1.59
8	7	1	90	2.60	90	1.68
13	8	1	65	1.90	-17.27	0.98
18	9	1	65	1.90	50	1
7	10	1	40	2.60	90	1.13
4	11	1	90	2.60	10	0.99
16	12	1	65	1.90	50	1.35
20	13	1	65	1.90	50	1.31
10	14	1	107.04	1.90	50	1
1	15	1	40	1.20	10	0.23
19	16	1	65	1.90	50	0.52
6	17	1	90	1.20	90	2.05
2	18	1	90	1.20	10	1.45
11	19	1	65	0.72	50	1.56
9	20	1	22	1.90	50	0.56

Table 5: Summary of pectin extraction

	Pectin Extraction at pH 2.5, 90°C, extraction time-90 min.					
	Citric Acid		HNO_3		HCl	
Acid used	Gooseberry	Strawberry	Gooseberry	Strawberry	Gooseberry	Strawberry
Pectin (gm)	0.274 gm	0.02 gm	0.984 gm	0.05 gm	0.732 gm	0.03 gm
Pectin Yield (%)	5.48 %	0.667 %	19.68%	1.667 %	14.64 %	1%

Experimental Design and Statistical Analysis: Response surface methodology and Central Composite Design were used to standarise the extraction parameters. Experimental values obtained for pectin yield (%) for gooseberry and strawberry pomace are summarised in Table 3 and Table 4 respectively. The result shown in 3-D graphs (Figure 1 for Gooseberry pomace and Figure 2 for strawberry pomace) which indicate that with the increase in temperature, extraction time and pH, the yield of pectin increased. The expected value of pectin yield obtained using RSM were compared with observed values. Both these values were approximately very close to each other. This indicates that the response surface methodology validate the experimental data.

Pectin Extraction: Pectin was extracted from Gooseberry and strawberry pomace using Citric acid, HNO3 and HCl and yield of the two were compared (Table 5). The results were expressed as percent yield and are presented in Figure 3. Extraction with HNO3 resulted in maximum yeild. In gooseberries, the percentage yield of pectin was 19.68% with HNO_3, 14.64% with HCl and 5.48% with citric acid while in strawberries yield of pectin was 1.67% with HNO3, 1% with HCl and 0.67% with citric acid. Percentage yield of pectin at pH 2.5 from Kinnow peels, Musambi peels, Malta peels and Feutral peels was 14.01%, 18.50%, 15.29% and 18.64% respectively [16]. Pectin yield from of Kaffir Lime ranged from 25.9%-61.80 [17]. Pectin yield from Apple, Bananas, Raspberries and Oranges was 2.36% respectively [18]. Pectin yield from passion fruit peel was 10%-70% at pH 1.2-2.5 [13]. Pectin of Gooseberry pomace extracted with HNO3 and HCl while pectin of Strawberry pomace extracted with HNO_3 and Citric acid were further characterized for its equivalent weight, methoxyl content, anhyrouronic acid content and degree of esterification.

Characterization of Pectin

Equivalent Weight of Pectin: Extracted pectin was characterized for different parameters. Equivalent weight was determined by method of AOAC (AOAC 1998) and results are shown in Figure 4. In case of gooseberries, pectin extracted with HCl had relatively higher equivalent weight of 1851.85g as compared to that extracted with HNO_3 and Citric acid. In case strawberries, pectin extracted with Citric acid had more equivalent weight of 675g as compared to that extracted with HNO3 and HCl. Equivalent weight was used for determination of anhydrouronic acid content and degree of esterification.

Methoxyl Content: In gooseberries, pectin extracted with HNO_3 had higher methoxyl content (5.022%) as compared to that extracted with HCl (1.178%) and Citric acid(0.285%) whereas in case of strawberries, pectin extracted with citric acid had 3.41% methoxyl content which is more as compared to that of HNO3 and HCl (Figure 5). Methoxyl content is an important factor in controlling the settling time of pectin, sensitivity to polyvalent cations and its usefulness in preparation of low solid gels, films and fibres. Low methoxyl pectins (< 50% esterified) form thermoreversible gels at low pH (3-4.5) in the presence of calcium ions whereas high methoxyl pectins rapidly form thermally irreversible gels at low pH (<3.5) in the presence of sufficient sugars such as sucrose. Pectin may have low or high methoxyl content. Lower the methoxyl content, slower pectins set. Both gooseberries and strawberries are low methoxyl pectins. Low methoxyl pectin are useful and are being successfully used in making low sugar jams and jellies. Methoxyl content is an important factor in controlling setting time of pectin sensitivity to polyvalent cations and in making low solid gels, films and fibres [12].

Anhydrouronic Acid Content: Anhydrouronic acid content of the extracted pectin is shown in Figure 6. In gooseberries anhydrouronic acid content of pectin extracted with HCl, HNO_3 and Citric acid was 76.38%, 39.78% and 19.25% respectively. In strawberries, anhydrouronic acid content of pectin extracted with citric acid was 22.88% which was more than that of extracted with HNO_3 (14.43%) and HCl (34.65). The AUA content indicates purity of extracted pectin. To be pure, pectin content should not be less than 65%. Studies suggest that Pectin extracted from gooseberries using HCl was pure. Extraction using Nitric acid did not give pure pectin in both fruits, indicates that pectin from both fruits is not pure.

Figure 1: Gooseberry

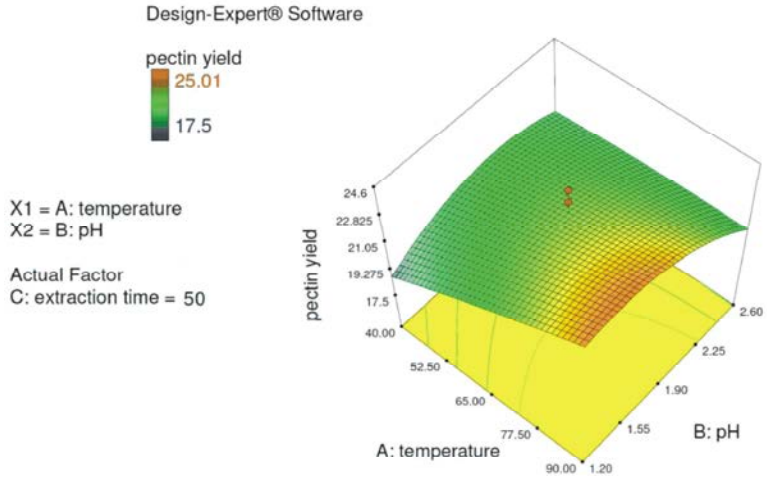

Fig. 1(A): Effect of temperature and pH on pectin yield

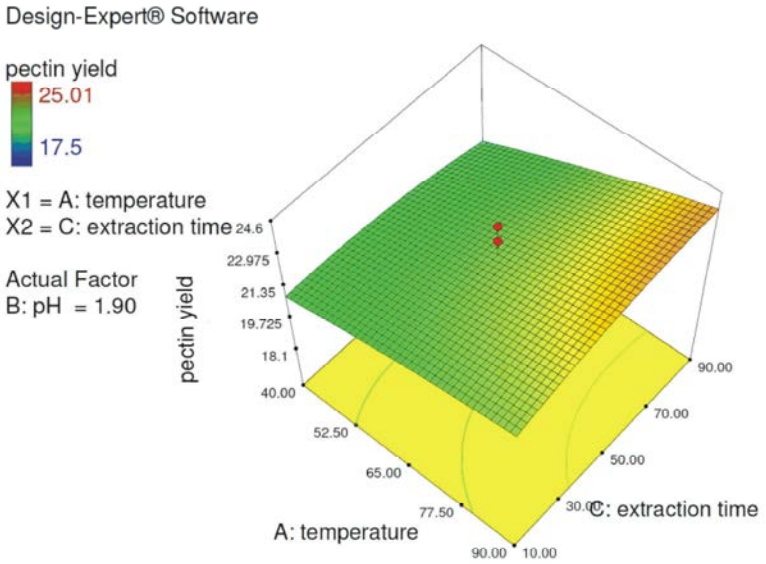

Fig. 1(B): Effect of temperature and extraction time on pectin yield

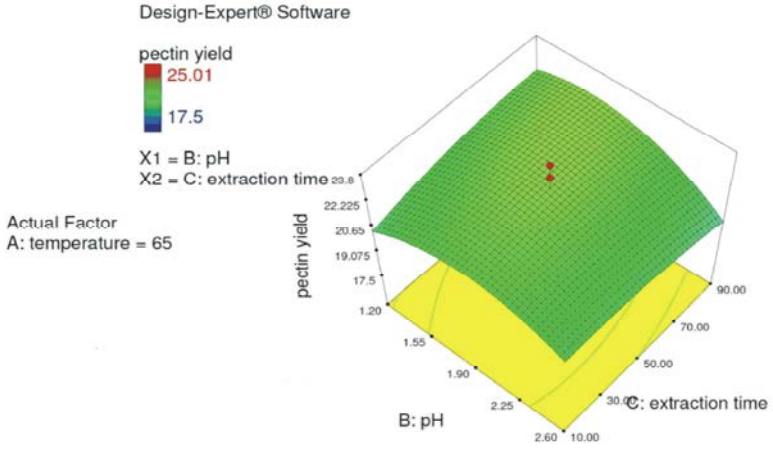

Fig. 1(C): Effect of pH and extraction time on pectin yield

Figure 2: Strawberry

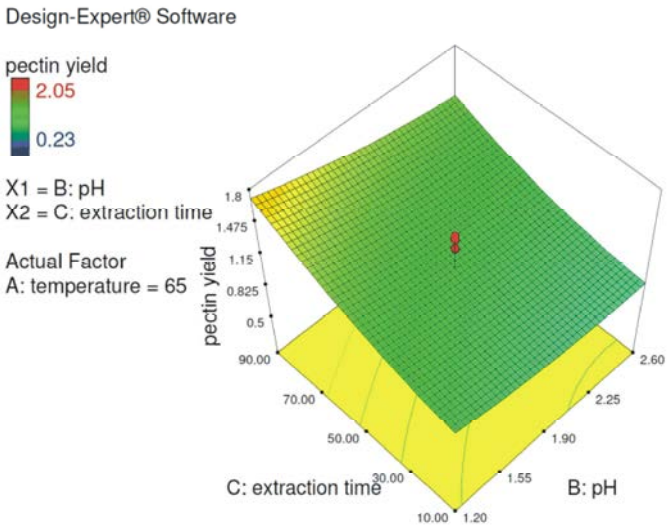

Fig. 2(A): Effect of pH and extraction time on pectin yield

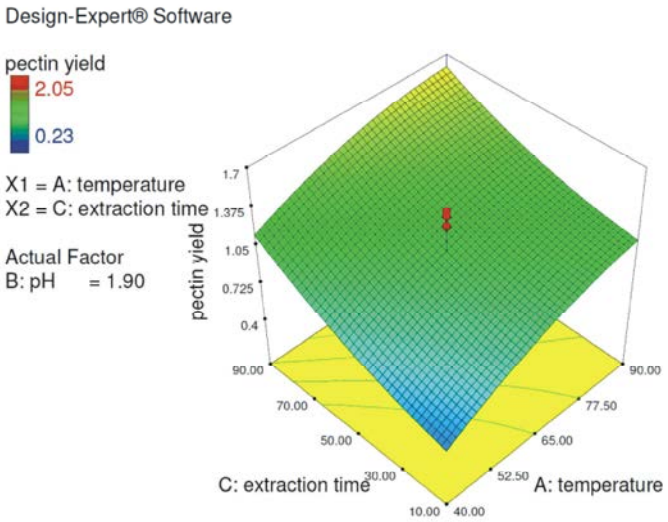

Fig. 2(B): Effect of extraction time and temperature on pectin yield

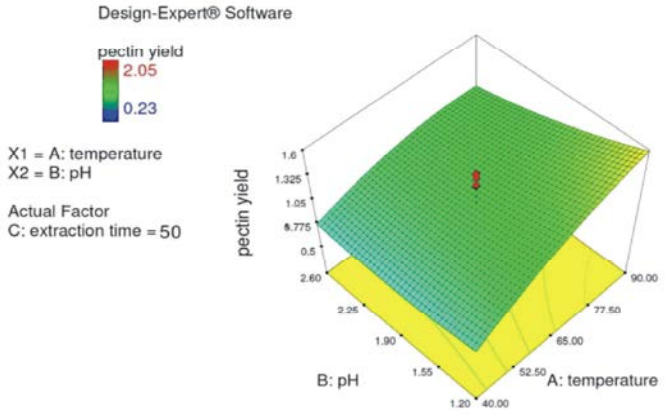

Fig. 2(C): Effect of pH and temperature on pectin yield

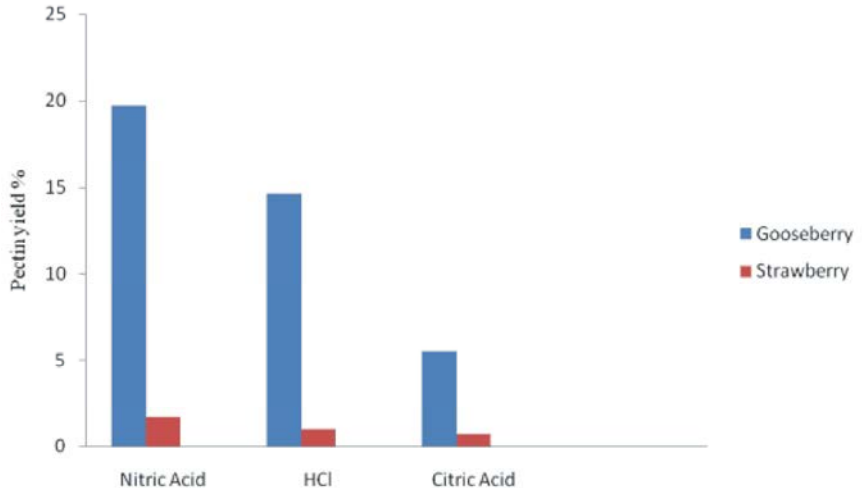

Fig. 3: Pectin yield % in fruit pomace

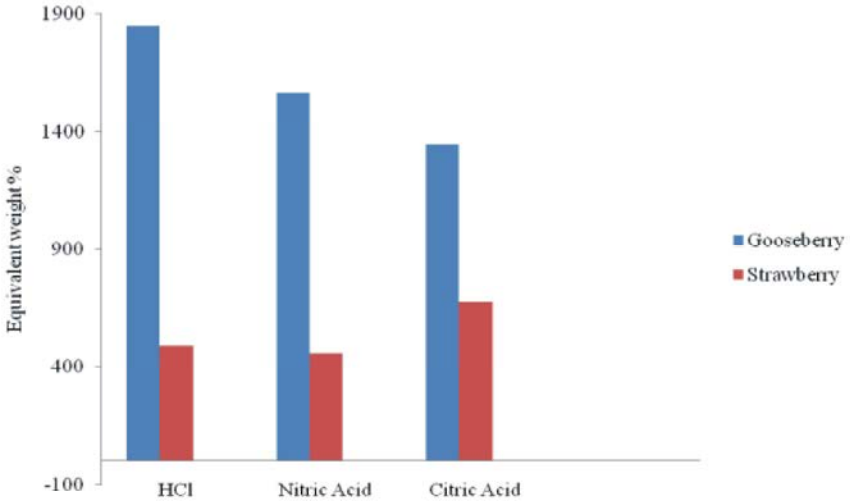

Fig. 4: Equivalent weight of fruit pomace

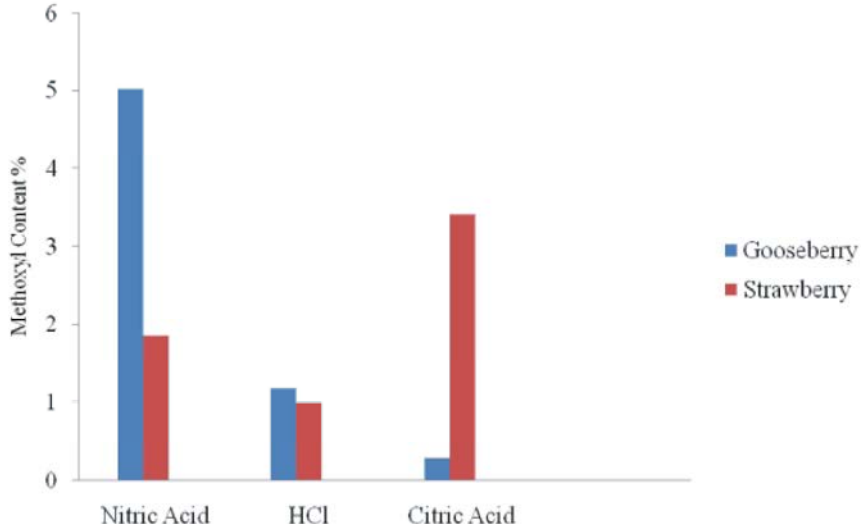

Fig. 5: Methoxyl content of fruit pomace

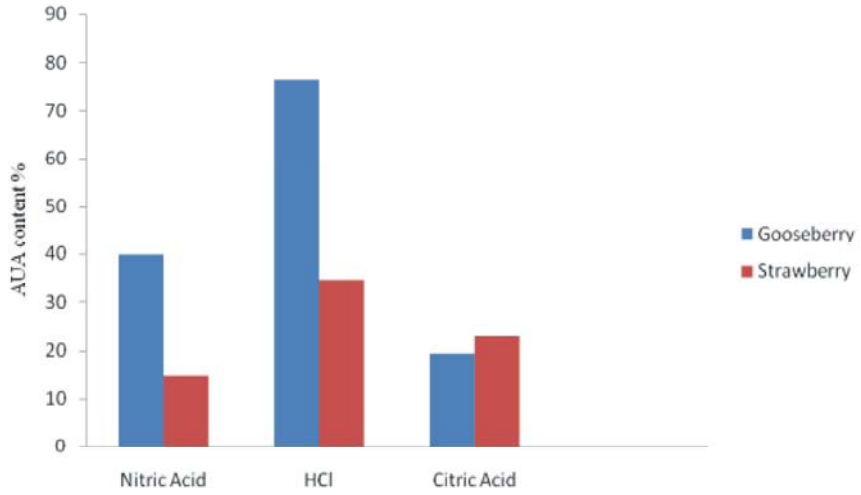

Fig. 6: AUA content of fruit pomace

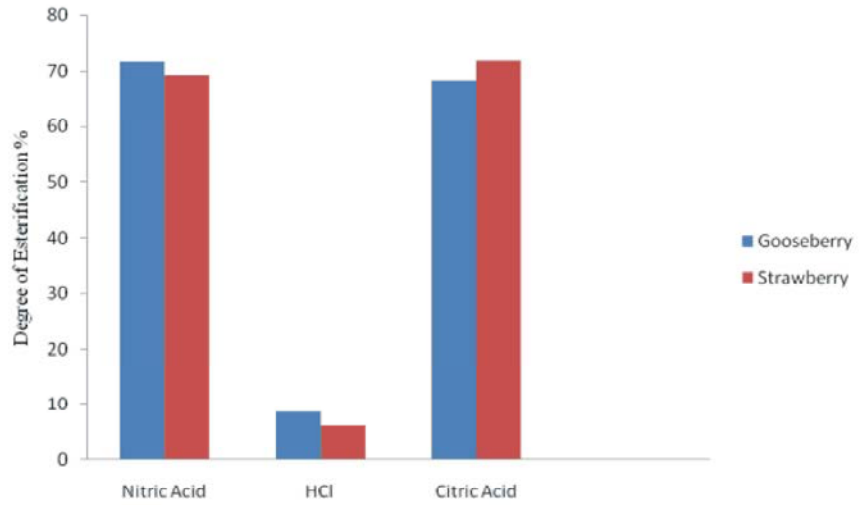

Fig. 7: Degree of esterification of fruit pomace

Degree of Esteification (DE): In both the fruit pomace, degree of esterification (DE) of extracted pectin is shown in Figure 7. In case of gooseberry pectin extracted with HNO_3 had 71.68% DE whereas that of extracted with HCl and Citric acid was 8.76 and 68.14% respectively. On the other hand, DE of pectin extracted from strawberries using Citric acid, HCL and HNO_3 was 71.88%, 6.24% and 69.28% respectively. The high DE% value (>50%) suggest that pectin extracted with HNO_3 and citric acid from both fruits pomace may be a source of pectin in food industry because pectin with high %DE have good gelling properties. On the basis of %DE, pectin are classified into high ester (with DE more than 50%) and low ester (with DE less than 50%). High ester pectin is stabilized by hydroform and hydrophobic interactions thus form a 3-dimensional molecular network and low water activity gel is formed. In low ester pectins, ionic bonds are formed between calcium ions and ionized carboxyl groups of galacturonic acid. So calcium ions are required to form gel.

Pectin from gooseberries and strawberries pomace may be classified in high ester group and is more valuable as it is expected to have good gelling properties.

CONCLUSION

Pectin extraction is a muti-stage process in which hydrolysis and extraction of pectins take place under influence of different factors viz., temperature, pH and extraction time. By using response surface methodology, the satisfactory condition of operating variables were obtained to extract maximum pectin from fruit pomace. Gooseberries and Strawberries are rich source of pectin.

Pectin was extracted from fruit pomace using different acids i.e. Citric acid, Nitric acid and Hydrochloric acid. Nitric acid was most effective extractant in solubilizing and releasing pectin. Successful extraction of pectin provide potential benefits for industrial extraction of pectin from both economically and environmentally point of view. High degree of esterification (>50%) of extracted pectin suggest that both Gooseberry and Strawberry pectin can be used in food industry. Pectin from different sources differ in their gelling ability. Suitable pectin can be selected for jam and for jellies or for higher sugar confectionary jellies providing both Gooseberry and Strawberry are promising fruits for this purpose.

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