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Agro-Industrial Solid Biodegradable Waste as a Source of Pectin and Feed for Livestock Farm-An Appraisal

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A B S T R A C T

Pectin is becoming increasingly popular as an effective additive in food industry because of its gelling, stabilizing, thickening and emulsifying properties. From the entire production of apple across the world about 71 per cent of apple is consumed as fresh apple while about 25-29 per cent is transformed into value -added products of which 65 per cent are processed into apple juice concentrate (AJC) and the balance quantity into other products which include packed natural ready-to-serve (RTS) apple juice, apple cider, wine and vermouth, apple purees, jams and dried apple products etc. The conventional processing of this fruit for juice, cider and concentrate preparation removes 75 per cent of fresh weight as juice and remaining 25 per cent as solid waste, known as apple pomace that is considered to be rich source of sugars (17.35%), pectin (16.95%) and crude fibre (16.16%) with a very high biological oxygen demand i.e. 240 -19000 mg/l . Apple pomace could also be utilized directly as animal feed or for the extraction of value-added products like pectin. Acids, alkalines and enzymes treatments have been employed to extract pectin from various sources, but pectin isolation is mainly predominated by the conventional acid hydrolysis of plant raw material and extraction with pectolytic enzymes. Pectins are industrially obtained from apple pomace and citrus peels in a chemical way with strong acids such as oxalic, hydrochloric, nitric and sulphuric acids which are regarded as conventional acid extraction methods. Enzymatic extraction has been conducted with polygalacturonase, hemicellulose and cellulase, protease and microbial mixed enzymes. Also, a number of methods have been used to extract pectin like ultrasonic, autoclave, microwave and extrusion assisted treatments from agricultural by -products such as apple pomace, sugar beet pulp and orange peels. The commercial production of apple fiber powder from the pomace after extraction of juice has been practiced by certain companies in abroad (Tree Top Inc, Selah, W.A.), However, in India technology for extraction of pectin on commercial scale from apple pomace is yet to be developed. The present reviews critically analyse the Extraction, Characterization and Applications of pectin obtained from apple pomace.

Introduction

Apple pomace being biodegradable in nature with high bio-chemical oxygen demand (BOD) and its direct disposal into the environment causes pollution (Kaushal *et al.*, 2002). Approximate proportions of various morphological constituents of apple pomace are pulp (54%), peel (34%), seeds (7%) seed core (4%) and stalks (2%). Chemically, the

Pomace is known to contain 66.4–78.2 % moisture, 26.4 % dry matter (DM), 9.5–22.0 % carbohydrates, 4.0 % proteins, 3.6 % sugars, 6.8 % cellulose, 0.38 % ash, 0.42 % acid and 8.7 mg/100 g calcium on wet weight basis (Vasil'ev *et al.,* 1976; Sun *et al.,* 2007). Joshi and Attri (2006) reported that apple pomace contained 48.00 % total sugar, 5.80 % crude protein, 14.70 % crude fibre, 18.50 mg/100g vitamin C and total 1.82 % ash on dry weight basis. Owing to high carbohydrate content, apple pomace is used as a substrate in a number of microbial processes for the production of organic acids, enzymes, single cell proteins, ethanol, low alcoholic drinks and pigments etc. (Bhushan *et al.,* 2008). Pectic substances in fruits were discovered by the French chemist Louis Nicolas Vauquelin in 1790 in tamarind fruit (Vauquelin, 1790). The term "pectin" was introduced by Henry Braconnot in 1825 from the Greek word 'pektikos' meaning to congeal or solidify (Braconnot, 1825). Pectic polysaccharides, localized in the primary cell wall and middle lamella in all higher plants, are responsible for different physiological processes (Knox, 2002). In the cell walls they serve as one of the main agents cementing the cellulose fibrils and may be linked covalently to other polymers. Intracellular pectins provide the channels for passage of nutrients and water (Tamaki *et al.,* 2008). Apple pomace is rich source of pectic substances (Fox, 1984).

Pectin is defined as a mixture of heteropolysaccharides. The polysaccharide structure is based on (1→4)-linked α-D-Galacturonic acid, interrupted by L-rhamnose residues with side chains of neutral sugars, mainly L-rhamnose, L-arabinose and D-galactose (Akhtar *et al.,* 2002). Galacturonic acid residues can be partially esterified by methanol on carboxyl group and by acetyl on the secondary hydroxyls (Willats *et al.,* 2006). Pectin forms gels under certain conditions and gelling mechanism is dependent on the degree of methoxylation (DM). Pectin is divided into high methoxyl pectin with DM> 50 per cent pectins and low methoxyl (LM) pectins with DM < 50 per cent (Strom *et al.,* 2007). Pectin with DM> 50 per cent form gels in the presence of high sugar concentration usually sucrose and fructose and low pH, whereas pectin with DM < 50 per cent forms gels in the presence of divalent ions (Fu and Rao, 2001).

Important sources of pomace

Kertez (1951) recommended Apples, citrus fruits, sugar beet and sunflower heads for pectin production. Commercially, pectin are primarily extracted from lime peel, guava extract, apple pomace (Chakraborty and Ray, 2011), orange (Braddock, 2004), cocoa husk (Mollea *et al.,* 2008), sunflower heads (Matora *et al.,* 1995), beet and potato pulp (Turquois *et al.,* 1999).

Particle size of raw material

Canteri-Schemin *et al.,* (2005) recorded 14 per cent higher pectin yield when the particle size was larger than 106 µm and smaller than 250 µm of apple pomace flour (9.73 %) as compared to apple pomace without grinding (6.13%). Higher yield was due to more protopectin in small particles of the substrate than in large ones. According to Attri and Maini (1996) pectin yield from galgal (*Citrus pseudolimon*) increased with decreasing the particle size of the peel. Later on El-Nawawi and Shehata (1987) found of 0.075 mm optimum particle diameter for extraction of pectin from Egyptian orange peel.

Extraction methods

According to Joye and Luzio, (2000) the method of pectin extraction basically involves the aqueous extraction of pectin

from the raw material, isolation , purification, and drying The yield of pectin usually depends upon the extraction conditions such as temperature, extraction time, pH, type of extraction solvents (Yeoh *et al.,* 2008) and drying method (Monsoor, 2005).

The most commonly used methods for the extraction of pectin are direct boiling, microwave heating (Garna *et al.,* 2007; El-Nawawi and Shehata, 1987; Liu *et al.,* 2006), ultrasonic (Panchev *et al.,* 1988), autoclave (Oosterveld *et al.,* 2000) and extrusion assisted extraction. The yield of pectin depends upon extraction conditions such as temperature, extraction time, pH and the types of extraction material (Fishman *et al.,* 1999; Liu *et al.,* 2006). Khan *et al.,* (2015) optimized temperature of 70^{o}C and time 30 minutes the most suitable papameters for pectin extraction from citrus peel. whereas earlier, Chakraborty and Ray (2011) optimized extraction time of 20 to 60 minutes and temperature of 80 to 100^{o}C and pH 1.4 to 2.6 for extraction of pectin from various sources.

Azad *et al.,* (2014) reported higher yield of pectin with distilled water having low ash content as compared to other solvents. Mollea *et al.,* (2008); Pinheiro *et al.,* (2008) and Fishman *et al.,* (2006) reported that pectin can be well extracted in an acidic aqueous medium with extraction solvents such as water with mineral acids like nitric, hydrochloric or sulphuric, phosphoric and citric acids. Canteri-Schemin *et al.,* (2005) recorded lowest pectin yields with phosphoric and malic acids while citric acid had the highest average yield of pectin (13.75%). Zhang and Taihua (2011) reported that high methoxyl pectin can be extracted by using water or mineral acids whereas hydrochloric acid, nitric, sulphuric and phosphoric acids can generally be used for low methoxyl pectins. Rascón-Chu *et al.,* (2009) assessed low quality 'Golden Delicious' apple fruits for acid-extraction of pectin with 6 per cent citric acid recovered pectin yield of 16 per cent. Whereas Pagan *et al.,* (1999) observed that pectin can be extracted from banana peels, pomegranate peels, pomace of potato and peaches using aqueous acidic solution in a magnetic thermostatic stirrer. Yapo and Koffi (2006) found that the rind of yellow passion fruit can be utilized for pectin extraction by using ammonium oxalate in addition to the dilute acid solutions. Attri and Maini (1996) tried various mineral acids and organic acid extractants for pectin extraction from galgal (*Citrus pseudolemon*) peel and found that maximum pectin yield of 15.26 per cent on dry weight basis was obtained with 0.1 N HCl and recommended mineral acids at lower pH as better extractant for maximum recovery of pectin than at higher pH. Chan and Choo (2013) extracted pectin from cocoa husks using water, citric acid at pH 2.5 or 4.0, or hydrochloric acid at pH 2.5 or 4.0 and reported that highest yield of pectin (7.62%) was obtained by using citric acid at pH 2.5 [1:25 (w/v)] at 95^{o}C for 3.0 h. However highest uronic acid content (65.20%) in the pectin was obtained by using water [1:25 (w/v)] at 95^{o}C for 3.0 h. Marcon *et al.,* (2005) extracted pectin from apple pomace with 5 per cent (w/v) citric acid as extracting agent with varied time intervals (30, 50 and 80 min) and temperature (50, 75 and 100 °C), the maximum pectin yield (16.8 %) was obtained by using higher temperatures (100 °C; 80 min) and more time. Canteri-Schemin *et al.,* (2005) extracted pectin from apple pomace aiming at establishing the optimum conditions for acid extraction using citric acid. Kalapathy and Proctor (2001) extracted pectin from soy hull using hydrochloric acid and the highest yield obtained was 28 per cent. Levigne *et al.,* (2002) used sugar beet as substrate for pectin extraction. They studied the effect of nitric and hydrochloric acid on pectin yield and found that different kinds of pectin can be

obtained with good yields at pH 1. Sohair *et al.,* (1987) extracted pectin from Egyptian orange peels using hydrochloric acid as the extracting agent, the maximum yield obtained was in the range of 21 to 30 per cent. A comparative study of pectin yield from orange peels using different acids was carried out by Shakila Bano *et al.,* (2012) and they found that during the drying process there was a considerable difference in the pectin yield using different acids. Sayah *et al.,* (2014) recommended the process of EO extraction then pectin isolation using citric acid hydrolysis techniques for Moroccan orange peels. Garna *et al.,* (2007) reported that pectin extraction by using chelating agents influence the pectin functionality as residual amount of chelating agents remained in the final pectin sample. May (1990) observed that extraction of pectin by alkali cause a decrease in the degree of esterification and also reduced the length of pectin chains by β-elimination. The addition of ammonia to pectin at low temperature result in the conversion of some ester groups into amide groups.

Microwave heating extraction takes less than 15 min to extract a satisfactory amount of pectin (Fishman *et al.,* 2000) and more effective in terms of pectin yield and gives better quality products (Kratchanova *et al.,* 2004). Fishman *et al.,* (2006) and Yeoh *et al.,* (2008) reported that microwave assisted extraction from sugar beet pulp and orange peels under different operating conditions could extract pectin in minutes rather than hours as required by conventional heating. Koh *et al.,* (2014) investigated the effect of power level of microwave assisted extraction (450 W, 600 W and 800 W) on yield and quality of extracted pectin from jackfruit rinds and compared with water-based extraction method at 90°C for extraction duration of 10 min and 1 hr, respectively. High yield of pectin was obtained from microwave assisted extraction (16.72-17.63%) as compared to conventional extraction (14.59%). Fishman *et al.,* (2006), extracted pectin from lime flavedo, albedo and pulp by employing microwave-assisted extraction under pressure with heating time from 1 to 10 min. The heating time of 3 min was optimized, with average molar mass ranged from about 310,000 to 515,000 Da, and average intrinsic viscosities ranged from about 9.5 to 13 dL/g. Fishman *et al.,* (2003) reported lower pectin yield from flash extraction as compared to commercial method of citrus pectin extraction but he observed that pectin extracted was comparable with microwave heating under pressure from orange albedo by steam injection under pressure.

Although the use of strong acids provides high extraction yield and time saving advantages, but it can cause serious environmental problems such as disposal of acidic waste water and also play a negative role in commuted preference. Thus several thermal or mechanical treatments have been applied to extract pectin including ultrasound (Panchev *et al.,* 1988) autoclaving (Oosterveld *et al.,* 2000; Hwang 2003) and subcritical water extraction (Tanaka *et al.,* 2008). Panchev *et al.,* (1988) and Bagherian *et al.,* (2011) found ultrasound assisted extraction less expensive process that uses acoustic energy and solvents to extract specific compounds from various plant matrices in relatively shorter times than with conventional extraction techniques with a higher yield**.** Ghafoor *et al.,* (2009) reported that the increase in extraction is mainly due to the passage of ultrasound wave to the solvent which results in acoustic cavitations. Bai *et al.,* (2015) optimized conditions for Jujube pectin extraction with extraction parameters i.e. liquid-solid ratio (5-15), pH (1.5-2.5), ultrasonic time (10-20 min) and microwave irradiation time (40-60 sec) and found best conditions for Jujube pectin extraction 10.03 mL/g of LSR, 1.97 of pH of sulphuric and

17.66 min of ultrasonic time and 52.73 sec of microwave irradiation time with the pectin yield of 1.95±0.0 per cent. 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). The enzyme concentration (1.05 ml/kg) of celluclase 1.5L resulted in the highest pectin yield and also exhibited highest viscosity.

The use of either a low level or a high level of enzyme showed lower pectin yields as the high enzyme concentration used could have resulted in greater pectin hydrolysis whereas the low enzyme concentration could have resulted in a low yield because insufficient enzyme was used for pectin extraction. Yuliarti *et al.,* (2015) carried out the studies on extraction of pectin from gold kiwifruit from two different stages of maturity i.e. early harvested fruit and main harvested fruit isolated by three methods (acid, water and enzymatic). Pectin extracted from main harvested fruit was higher in galacturonic acid content and weight-average molecular weights compared with EHF pectins. Enzymatic treatment gave the highest yield but lowest in molecular weight. The study demonstrated that water extracted pectin exhibited the highest viscosities, suggesting that pectin obtained from water extraction method was least detrimental to the pectin molecule in the native state. Natural catalysis with endo-polyglacturonase (Conteres-Esquivel *et al.,* 2006), hemicellulase (Shkodina *et al.,* 1998) with protease (Zykwinska *et al.,* 2008) and microbial enzymes (Pichkina *et al.,* 2008) have been used so far for extraction of pectin from different sources. Panouille *et al.,* (2006) reported that enzymes can extract pectins with a higher yield for a smaller mass than acid extracted pectins. They further reported that the use of cellulase in the isolation of pectin from chicory roots and cauliflower. These researchers indicated that cellulase was effective in hydrolyzing the cellulose from cell wall. Pumpkin pectin has been reported to have lower gel strength (10 KPa) when extracted by enzymes prepared from *Aspergillus awamori* (Pichkina *et al.,* 2008).

The technology of plant extraction using direct induction heating assisted by magnetic field has been investigated only in the case of producing parietal fractions and fractions of secondary metabolites from wheat straw, madder roots and caraway seeds (Lagunez - Rivera and Vilarem, 2007). Zoumbia *et al.,* (2014) used electromagnetic induction for extraction of pectin from citrange albedo. They compared the protopectin of the extracted pectins with those obtained by using conventional heating. They found the time required for the extracting process was significantly reduced from 90 min for conventional method to 30 min for electromagnetic induction method as well as observed significant influence on the structural properties of pectin.

Concentration of pectin extract

The concentration of extract is done in order to reduce the volume of pectin extract so as to economize the use of precipitating agents. Kulkarni and Vijayanand (2010) recommended vacuum concentration of pectin extract for reducing the amount of alcohol required for the precipitation of the pectin

extract. Muminov (1997) has mentioned, that the clarified extract of pectin should be concentrated in vacuum evaporator at 60-70^{o}C for reducing the amount of ethanol required for the precipitation of pectin without any significant effect on the quality of pectin.

Precipitation of pectin extract

Ihl *et al.,* (1992) studied the recovery of pectin from apple pomace (*cv.* Pippin) by using different precipitating agents and reported higher pectin yield of 7.2 per cent with ethanol, while lower pectin yield of 1.11 per cent with 2 per cent $AlCl_3$, 2.38 per cent with 3 per cent $AlCl_3$ and 2.8 per cent with 4 per cent $AlCl_3$. On the basis of high yield and acceptable properties of pectin overall precipitation with ethanol was recommended.

Attri and Maini (1996) standardized process for maximum recovery of pectin from Galgal (*Citrus pseudolimon* Tan.) peels and found ethanol precipitation better than aluminium-chloride precipitation on the basis of quality parameters of pectin. Muminov and Salonov (1992) has compared various precipitants i.e. polyvalent metal salts i.e. aluminium chloride, calcium chloride and copper sulphate and organic precipitants i.e. ethanol, isopropanol, acetone for pectin precipitation from cotton valves and recommended ethanol as an effective precipitating agent. Zhang and Liu (2000) found alcohol precipitation better for pectin extraction from pomelo (*Citrus grandis*)

Similarly Chen and Li (1994) reported for apple pomace pectin. Bhardwaj *et al.,* (2014) compared ethanol ratio 1:1, 1:2 and 1:3 and found maximum pectin yield with 1:1 ethanol ratio. Arthey and Ashurst (1996) reported that pectin precipitation is governed by the addition of suitable amount of alcohol into pectin solution, which can increase the yield.

Quality evaluation of pectin

Ash content

On the basis of ash content Ranganna (1997) has differentiated the pectin as high ash content pectin (10.69 per cent ash content) and low ash content pectin (0.76 per cent ash content). Ramli and Asmawati (2011) extracted pectin from cocoa husk with ammonium oxalate and found highest percentage of ash content of pectin 12.93 per cent with at the extraction time of 120 min. Virk and Sogi (2004) recorded ash content of 1.44 percent from apple pomace peel powder pectin extracted with citric acid, lower ash content in commercial pectin was 1.16 per cent. Johar *et al.,* (1960) found that the ash content of pectin extracted from golden delicious apple pomace was 1.84 percent while Pruthi *et al.,* (1961) observed ash content of 0.45 per cent and 0.50 per cent for mandarin pomace pectin and mandarin peel pectin respectively. Dang (1968) reported ash content of 4.79 per cent in papaya pectin, 0.70 per cent in Assam lemon pectin and 1.72 per cent in galgal pectin. Baississe *et al.,* (2010) observed that ash content of apple and apple pomace pectin varied between 10 and 16 per cent respectively. They observed that ash content increases with increasing the precipitation, pH value and the concentration of aluminium sulphate. However with the use of organic solvents such as alcohol, lower ash content was recorded and it can be by increasing the lessivassion of the impurities during pectin precipitation. Mohd *et al.,* (2012) found that dragon fruit pectin contained lower ash content with the acid than water extracted pectin. Low ash content is good for gel formation. The maximum limit of ash content for good quality gel criteria is 10 per cent. Zouambia *et al.,* (2014) observed similar ash content in the pectin of citrange fruit following electromagnetic induction heating and conventional heating of

extraction. Yuliarti *et al.,* (2015) found highest ash content from gold kiwifruit purified pectin samples extracted from the early harvested fruit by the enzymatic method. They found that the enzymatic method could potentially hydrolyze the cell wall material resulting in the release of more non pectin components. However lowest ash content were obtained in water extracted main harvested fruit pectin indicating the purest form of pectin.

Equivalent weight

Equivalent weight of pectin is a measure of the total content of free galacturonic acid (not esterified) in the molecular chains of pectin (Ranganna 1997). The equivalent weight of pectin was 368±32 to 1632± 13 in lemon pomace (Azad *et al.,* 2014), 833.33 to 1666.30 in apple pomace (Kumar and Chauhan 2010); and 1088.9 in grapefruit peel and 818.8. in guava pomace (Hafeez *et al.,* 2014). Ramli and Asmawati (2011) reported that ammonium oxalate produced pectin with higher equivalent weight (580.81) compared with acetic acid (565.49) from cocoa husks and reported that the increase and decrease of equivalent weight might be dependent upon the amount of free acid. Mohd *et al.,* (2012) worked upon characterization of pectin from dragon fruit using various extraction conditions and found maximum equivalent weight with acid extraction as compared to other methods of extraction. Kumar and Chauhan (2010) reported that equivalent weight of apple pomace pectin ranged from 833.33 to 1666.30. Virk and Sogi (2004) reported that equivalent weight of pectin extracted from apple peel was 652.5 and commercial pectin was found to be 1030.9. Sharma *et al.,* (1985) recorded that equivalent weight of pectins extracted from golden delicious apple pomace and golden delicious partially ripe apples were 1384.0 and 1027.7 respectively. Shaha *et al.,* (2013) reported 735.4 equivalent weight of pectin from kaffir lime peel dried in the microwave using hydrochloric acid extraction at 90^{o}C.

Methoxyl content

Methoxyl content is defined as the number of moles of methyl alcohol in 100 mol galacturonic acid. Methoxyl content is an important quality factor in controlling the setting time of pectins and the ability of the pectin to form gels (Constenla and Lozano, 2003). Based on the number of ester groups, the type of pectin can be categorized as low methoxyl pectin where the ester groups were less than 50 per cent and high methoxyl pectin when ester groups are more than 50 per cent. Methoxyl content present in pectin extracted from the peel of mango (7.33%), banana (7.03%), pomelo peel (8.57%) lime (9.92%) (Madhav and Pushpalatha, 2002), passion fruit (8.81% to 9.61%) but higher than dragon fruit pectin (2.98% to 4.34%) (Ismail *et al.,* 2012). Azad *et al.,* (2014) isolated and characterized the pectin extracted from lemon pomace during ripening and observed higher methoxyl content of 10.25 followed by mature and over ripe fruits. The methoxyl content decreased with increase of maturity. Due to ripening the sugar content of fruits increased and methoxyl content decreased (Sirisakulwat *et al.,* 2008). Spreading quality and sugar binding capacity of pectin increased with increase in methoxyl content (Madhav and Pushpalatha, 2002). Hafeez *et al.,* (2014) compared the pectin extracted from wastes of guava and grapefruits and found significantly higher 10.55 per cent in grapefruit peel than that recorded for guava pomace 8.37 per cent. Virk and Sogi, 2004 extracted pectin from apple pomace peel powder with citric acid extractant and recorded 3.7 per cent methoxyl content. Jain *et al.,* (1984) and Sharma *et al.,* (1985) have reported that the methoxyl content of pectin extracted from golden delicious apple pomace and golden delicious

partially ripe apple as 8.73 per cent and 7.4 per cent respectively. The methoxyl content of pectin extracted from unblanched, washed and dried apple peel and pomace was 8.0 per cent (Chaliha *et al.,* 1963) and of blanched one was 8.74 per cent. Srirangarajan and Shrikhande (1977) have reported 8.25 per cent methoxyl content of pectin extracted from mango peel.

Anhydrogalacturonic acid (AGA) content

Anhydrouronic acid indicates the purity of the extracted pectin and its value should not be less than 65 per cent (Food Chemical codex, 1981). Kumar and Chauhan (2010) found 59.52 to 70.00 per cent of AGA in apple pomace pectin and commercial pectin respectively. Lo Scalzo *et al.,* (2005) obtained high AGA content at pH 1.5, this suggest that pectin AGA content increases with pH decrease and it is related to enzymatic activity decrease particularly polyglacturonase when optimum pH is 3.5 (Eskin 1990). Ferantoni *et al.,* (2006) found AGA between 53 and 75 per cent for apple pomace. Polysaccharides such as pectin show qualitative and quantitative variations due to fruit variety, stage of maturity, geographical origin, storage condition (Veberic and Stampar, 2005) and extraction parameters (Ferantoni *et al.,* 2006) while Ismail *et al.,* (2012) reported 61.72 per cent and 45.25 to 52.45 per cent. Low value of AGA means that the extracted pectin might have a high amount of protein. Virk and Sogi (2004) recorded anhydrouronic acid content of pectin extracted from apple pomace peel powder was 62.8 per cent. Jain *et al.,* (1984) and Sharma *et al.,* (1985) have reported that the anhydrouronic acid content of pectin extracted from golden delicious apple pomace and golden delicious partially ripe apple 74.1 per cent and 73.9 per cent respectively. Jain *et al.,* (1984) reported that pectin obtained from mango peel was reported to contain 61.1 per cent anhydrouronic acid. Lim *et al.,* (2012) recorded that combined physical and enzymatic extraction produced pectin with 54.5 per cent of galaturonic acid as compared to 72.3 per cent with chemically-extracted pectin. Chan and Choo (2013) reported that anhydrogalacturonic acid contents of the pectin extracted from cocoa husks ranged from 31.19–65.20 per cent. Rascón-Chu *et al.,* (2009) assessed that pectin extracted from low quality 'Golden Delicious' apple fruit presented anhydrogalacturonic acid content of 65 per cent.

Degree of esterification (DE)

It is defined as the percentage of the amount of D-galacturonic acid in which the carboxyl group passed through the process of esterification with ethyl alcohol and is important means to classify pectins. The degree of esterification varies with the fraction of raw material and precipitating agent. Massiot and Renard (1997) and Ferantoni *et al.,* (2006) recorded 2.20 to 4.40 methanol content in apple pectin. However Renard *et al.,* (1990) obtained highly methoxylated pectin from apples in general co-products resulting from fruit process contain pectinase, mainly pectin with low DE. Ptichkina *et al.,* (2008) stated that pectin with DE > 60% is suitable for use in the food industry. Degree of esterification is related to the rate of gel formation in food industry as it influences the quality of the gel (Pagan *et al.,* 1999). High degree of esterification means high gelation temperature, while preparing gels by the usual procedure of mixing hot ingredients and then solidifying by cooling. Daas *et al.,* (2001) reported that when the gel batch is cooled below the gelling temperature, gelation occurs after a delay, which is short with pectin of high degree of esterification and longer with pectin of lower degree of esterification. Rascón-Chu *et al.,* (2009) found that pectin extracted from low quality 'Golden Delicious' apple fruit by acid

extraction with 6 per cent citric acid presented an esterification degree of 57 per cent. Virk and Sogi (2004) found that degree of esterification of pectin extracted from apple pomace peel powder was 33.44 per cent. The degree of esterification of pectin extracted from golden delicious pomace and golden delicious partially ripe apple was 72.40 per cent and 56.9 per cent respectively (Jain *et al.,* 1984 ; Sharma *et al.,* 1985) whereas that of mango peel pectin was 76 per cent (Srirangarajan and Shrikhande, 1977). Ramli and Asmawati (2011) found that extraction time of 60 min produced pectin with higher degree of esterification (50.33%) than 120 min (49.41%) from cocoa husks, as longer extraction time might cause degradation process of methyl ester groups in pectin into carboxyl acid. Canteri-Schemin *et al.,* (2005) found that pectin extracted from apple pomace showed a degree of esterification of approximately 68.84 per cent. Lim *et al.,* (2012) found that the pectin extracted from Yuza (*Citrus junos*) pomace by combined physical and enzymatic extraction exhibited a higher degree of esterification (46 %) than chemically-extracted pectin (41%), which was confirmed by FT-IR analysis. Hafeez *et al.,* (2014) found significantly higher (89.23%) degree of esterification in grapefruit peel than that of guava pomace pectin (83.75%). Jams and preserves are of course the main use of industrially extracted pectins. High dissolved sugars and acid conditions ensure that chain-chain interactions dominate once chain-solvent interactions occur (Sharma *et al.,* 2006). Most chain-chain interactions in these systems are not based on electrostatic interaction and so the other hydrophobic and hydrogen bonding effects exert most influence. Azad and his co-workers (2014) found DE of lemon pomace pectin in the range of 33.59±0.17 and 79.51± 0.36 per cent. The premature and mature samples produced high degree of esterification 79.00±0.36 and 70.39±4.20 per cent respectively. Sotanaphun *et al.,* (2012) and Ismail *et al.,* (2012) reported 76.30 per cent DE in *Citrus maxima* and 31 to 52 per cent DE in dragon fruit. The lower DE might be attributed to the conversion of pectin into the protopectin which increase the sugars and makes the fruit softer (Bartley and Knee 1981; Redgwell *et al.,* 1997) during the maturation due to chemical reasons occurs at too low pH and too high temperatures. According to Sundar Raj *et al.,* (2012) DE actually depends upon species, tissues and stages of maturity.

Structure analysis

The infrared spectroscopy is a fast and convenient method for investigation of functional group of polysaccharides. Fourier transform infrared spectroscopic methods are useful tools in the structural characterization of natural and modified pectins as well as other plant cell wall. Polysaccharides as the contents of feruloyl groups, degree of methylation and amidation are estimated by using these spectroscopic methods (Synytsya 2003; Sinitsya *et al.,* 2000). The study of hydrocolloids usually focuses on chemical composition, linkage between the monosaccharide units, and the size and shape of the molecule. A complete analysis of hydrocolloids or polysaccharides is unsuitable for their rapid identification in food or raw materials because the whole procedure is rather costly and time consuming. Recently, Fourier transform infrared spectroscopy has become a well accepted method for the determination of food constituents since it achieves high analysis speed and requires little or no sample preparation. Fourier transform infrared spectroscopy often coupled with chemometrics used to study different quality attributes in many food samples including fruits, vegetables or beverages e.g. epicuticular wax of apple (Veraverbeke *et al.,* 2005), polymethoxylated flavone of orange oil residues (Manthey, 2006), vitamin C in

powdered mixture and liquid (Yang and Irudayaraj, 2002), must and wine analysis (Fernandez and Agosin, 2007) moreover, it has become an alternative method for sugar analysis (Bellon-Maurel *et al.,* 1995) in food such as mango juices (Duarte *et al.,* 2002), soft drinks and fruit juices (Ramasami *et al.,* 2004). Fourier transform infrared spectra are generally analyzed to confirm the identity of extracted pectins and to estimate their degree of esterification (DE). Kalapathy and Proctor (2001) and Wellner *et al.,* (1998) reported that Fourier transform infrared spectra shows the functional groups and structural information of different extracted pectins and the pure pectin in the region between 1,000^{-1} and 2,000 cm^{-1}. The interpretation of Fourier transform infrared spectra between 3600 and 2500 cm^{-1} shows broad, strong area of absorption which generally refers to O±H stretching absorption due to inter and intramolecular hydrogen bonds. The O-H stretching vibrations occur within a broad range of frequencies and indicate several features of a compound, including ``free'' hydroxyl groups stretching bands which occurs in samples in vapour phase and bonded O-H bands of carboxylic acid (Silverstein *et al.,* 1991). In the case of pectin samples, absorption in the O-H region is due to inter and intramolecular hydrogen bonding of the galacturonic acid polymer. Finer bands appearing at the longer end of the O-H region indicate overtones and combination of tones. Bands around 2950 cm^{-1} (3000-2800 cm^{-1}) refer to C-H absorption. These include CH, CH_2, and CH_3 stretching and bending vibrations. Typically, two moderately intense bands are observed in the C-H region of aliphatic compounds. In pectin samples, the C-H stretching and bending vibrations are seen, usually, as a band superimposed upon the broader O-H band that ranges from 2500 to 3600 cm^{-1}. In the case of esterified pectins, an O-CH_3 stretching band is expected between 2950 and 2750 cm^{-1} due to methyl esters of galacturonic acid. However, due to a large O-H stretching response occurring in a broad region (3600- 2500 cm^{-1}), the O-CH_3 activity is masked and is not a reliable indicator of methoxylation. Stronger bands occurring between 1760-1745 cm^{-1} and between 1640 and 1620 cm^{-1} indicate the ester carbonyl (C-O) groups and carboxylate ion stretching band (COO-), respectively. The bands representing ester carbonyl (1760- 1745 cm^{-1}) and free carboxylate groups (1640-1620 cm^{-1}) are important in the identification and quantification of pectin samples. Carboxylate groups show two bands, an asymmetrical stretching band near 1650-1550 cm^{-1}, and a weaker symmetric stretching band near 1400 cm^{-1}. In pectin, the weaker symmetric COO- stretching is followed by moderately intense absorption patterns between 1300 and 800 cm^{-1} collectively referred to as the ``finger print'' region for carbohydrates as it allows to identify the major chemical groups (ether R-O-R and cyclic C-C bond) in polysaccharides (Cerna *et al.,* 2003). These bands are usually difficult to interpret. The carbonyl bands at 1630-1650 and 1740-1760 cm^{-1} indicate the presence of free and esterified carboxyl groups, respectively (Gnanasambandan and Proctor, 2000). They further reported that pectin esterification degree was calculated by taking the peak areas values of the free carboxyl groups (1650 cm^{-1}) and esterified groups (1750 cm^{-1}) using the following equation

$$DE = \frac{\text{Area of esterified carboxyl groups}}{(\text{Area of esterified carboxyl groups} + \text{Area of non-esterified carboxyl groups})} X\ 100$$

The FTIR spectra showed functional groups and structural information of pectin in the region between 1000 and 2000 cm^{-1} (Kalapathy and Proctor, 2000; Wellner *et al.,* 1998). Cerna *et al.,* (2003) found that the FTIR spectroscopy in the region of 1200- 800 cm^{-1} wavenumber region can be used as a very reliable and quick tool for food

authentication of carbohydrate based additives.

Pectin gelation

Compression tests are suitable for determining several mechanical and rheological characteristics of different foods with respect to consumer perception. According to Szczesniak (2002) hardness and cohesiveness were defined as the force required for attaining a given deformation and the extent to which a material can be deformed before it ruptures, respectively. The gel structure is supported by soft colloidal system containing gelling agents, sugars, water and other components (Warnecke, 1991) where water acts as a plasticizer to aid gel formation (BeMiller and Whistler, 2009). The sugar co-solutes are not part of the polymer network, but they contribute to the formation and rheological behavior of confectionery gels (Morris, 1985) while the pH may affect gelation depending on the isoelectric point of the gelatin used among other factors (Edwards, 2000). Mitchell (1980) used experimental methods for investigating the viscoelastic behavior of gels and emphasized that the rupture tests are not the best for evaluating gels. However, with small deformation or compression tests better results can be obtained as compared with rupture tests. The gel strength and elasticity can be measured by TA-XT plus Texture Analyzer using probe number P/0.5R (diameter 126.26 mm, material delrin) with test speed 2 mm/s and distance 25 mm. The force should be given to the center of the gel sample. The gel strength is calculated by dividing force given with area of the probe (Marine Colloids, 1978). Rascón-Chu *et al.*, (2009) assessed the texture profile analysis of the pectin gels and recorded increase in pectin gel hardness from 10.2 to 20.4 g on increasing the pectin concentration from 2 per cent to 3 per cent (w/v) which is related to the polysaccharide chain aggregation phenomena and promoted as the increase in polysaccharide concentration. The rheological stability of pectin gels at 2 per cent and 3 per cent (w/v) was determined after 48 hours at 4^{o}C and it was found that after this period of storage, pectin gel hardness decreased from 10.2 to 8.2 and from 20.4 to 15.3 for the gels at 2 and 3 per cent (w/v). This rheological evolution could be due to an increase in pectin hydration as a result of a moderate acid hydrolysis of the pectin during storage. As a fact, HM pectin gels belong to the category of physically cross-linked gels whose three-dimensional structure is stabilized mainly by multiple hydrophobic interactions and hydrogen bonds in the junction zones of the polymeric network (Schmelter *et al.*, 2002).

Applications

Apple pomace as a source of pectin

According to Liu *et al.*, (2003) Pectin can be commonly utilized as a gel forming agent in food and pharmaceutical applications. Pectin is a heteropolysaccharide made up of hydrocolloids and has been known to have applications in food industry a as gelling agent , emulsifier and stabilizer (Winning *et al.*, 2007). Canteri-Schemin *et al.*, (2005) has also reported the use of Pectin in processing of foods such as marmalade, jam, sauces, juices, jellies, ketchups, syrups, concentrate and yoghurts etc besides medical preparation to stabilize the suspensions. According to Aina *et al.*, (2012) pectin has commercial uses as agglutinates in blood therapy and also thickening agent in canning of meats. Apple pomace and pectin are used for the development of soft drink, jam, Jelly, apple pomace sauce, apple pomace papad, toffee, slab, butter, bread, biscuits and cookies etc. by Johar *et al.*, (1960); Joshi *et al.*, (1995); Joshi *et al.*, (1996); Barwal (1996); Kaushal *et al.*, (2002) ; Madieta *et al.*, (2006) and Ivy

and Singh (2006)). Alwood (1903); Johar *et al.,* (1960); Yasuhito (2006) and Xiaoqiong *et al.,* (2009) have described that pomace may be used for preparation of fermented beverages viz vinegar, beer, cider, flow over alcohol and biohydrogen (bio-H_2). There is large variety of commercial pectins available in market, differing mainly in their degrees of esterification, to meet the requirements of different jams or similar fruit containing, sugar rich, highly viscous systems (Voragen *et al.,* 1995).

Pectin is widely used in the dairy industry, due to its stabilizing and thickening properties to prevent aggregation and precipitation of caseins, pectin is used in low acid (pH 3.5 - 4.2) heat processed milk products (Voragen *et al.,* 1995). Pectin can be used as a thickener in yogurts; as a water binder in stirred yogurts and as emulsifier and to provide fat-like mouthfeel in low fat yogurts. Pectin is also used in the production of fruit bases for yogurts to ensure uniform distribution of the fruit and for reducing colour migration from the fruit to the yogurt (May 1990; Voragen *et al.,* 1995).

Pectin is used in bakery industry to retain moisture and to improve volume, flexibility and softness in breads.

In frozen dough, pectin is used to delay the retrogradation of starch, while stabilizing the volume of the dough during freezing (Brejnholt, 2010). Pectin is used in many food products such as mayonnaise, salad dressing, tomato ketchup, protein foams and beverages due to its stabilizing and thickening properties (Pilnik and Voragen 1992). Pectin is commonly used in low fat foods as a fat replacer (Min *et al.,* 2010). Foods can be also coated with pectin and polyvalent cations prior to frying, to reduce the absorption of oil (Gerrish and Carosino 2001).

Apple pomace as animal feed

According to Rumsey (1978) apple pomace is equivalent to corn silage in total digestible nutrients content and rich in pectin, pentosans and ether extract. Further reported that inclusion of 17 per cent (dry matter basis) of apple pomace in diet of fistulated steers led to slight reduction of rumen pH and increased acetate to propionate ratio. Narang and Lal (1985) while evaluating some agro- industrial wastes as feed of 'Jersy' calves on the basis of body weight gains, live body measurements like height, length, breadth etc. and metabolic trials, concluded that apple pomace can safely be mixed in the feed of animals.

Conventional and unconventional by-products from the food processing industry have been frequently included in livestock diets (Denek and Can, 2006). Abdollahzadeh *et al.,* (2010) studied the effect of replacing alfalfa hay with Ensiled Mixed Tomato and Apple Pomace (EMTAP) on performance of holstein dairy cows and reported that the nutritional value of tomato and apple pomace improved when used Ensiled Mixed Tomato and Apple Pomace together with ratio of 50:50.

Ghoreishi *et al.,* (2007) reported that dry matter (DM) intake increased significantly when apple pomace was fed to dairy cows. Toyokawa *et al.,* (1984) stated that the milk yield was increased when apple pomace was mixed well with wheat bran, chopped alfalfa and milled rice bran (10% DM basis), ensiled and then fed to dairy cows.Apple pomace is traditionally utilized as cattle feed, but only a small fraction of apple pomace is used due to the rapid spoilage of the wet pomace (Bates and Roberts 2001). Apple pomace is considered as good source of nutrients due to the presence of high amounts of carbohydrate, pectin, crude fiber and minerals. Joshi and Sandhu (1994); Joshi and Sandhu (1996) have advocated innovative approach for the

recovery of the ethanol and the production of animal feed concomitantly from apple pomace. Bae *et al.,* (1994) compared a total mixed ration containing 39 per cent apple pomace with conventional feeds (control).

They observed that cows fed apple pomace mixed ration showed increased protein content but decreased lactose content in milk, when compared with cows fed the control diet while milk fat and solid not fat (SNF) were similar for both diets. Body weight of cows fed with apple pomace mixed ration was better as compared to cows under control feeding.

The feed cost per kg milk production was higher with apple pomace mixed ration but the gross income (calculated as total milk cost minus total feed cost) was higher with apple pomace mixed ration than the control. Abdollazadeh *et al.,* (2010) studied the effect of feeding mixed tomato and apple pomace on performance of Holstein dairy cows.

Higher digestibility and palatability along with lower dry matter content of mixed tomato and apple pomace feed increased when they were mixed and ensiled. Ghoreishi *et al.,* (2007) found that dry matter intake increased significantly when apple pomace was fed to dairy cows. According to Church (1988) and De valle *et al.,* (2006) apple pomace has considerable amount of pectin which rumen bacteria can use to produce acetate by fermentation and leading to proper condition for milk fat synthesis.

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