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Pectin, Pectic Oligosaccharides (POS) and its Prebiotic Potential

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Abstract—*As the world population keeps on exacerbating at a tremendous unpremeditated rate, so is the food waste generation. Same is true for fruit waste, which forms a substantial share of the whole food waste piling up. It also requires expeditious examination in terms of its effective utilization. In the pursuit of realising a potent path for fruit waste usage, Pectin and Pectic Oligosaccharides (POS) production is a very viable and profitable option as fruit waste is predominantly rich in this polysaccharide. The principal sources of this versatile carbohydrate comprise of apple pomace, citrus waste, sugar beet residue, olive pomace. Others include mango peels, passion fruit peels, tomato processing waste. Extraction methods are: fundamental acid-based extraction and novel enzymatic, subcritical water, ultrasound, microwave extractions. Depolymersation of pectin yields POS. In addition to all this, the present review also throws light upon the prebiotic potential of these oligosaccharides which even prove to be a better prebiotic candidate than their parent pectin. Use of POS as a prebiotic is still at a nascent stage and therefore, it offers new avenues for further research as well as for new product development.*

1. INTRODUCTION

World population is on the rise and with this ever-increasing population, the demand for resources is also increasing [1]. One such resource which is bearing the brunt of this growing population is food [1]. In the quest of producing more food for the growing population, more food waste is being generated. While there is no ambiguity in the fact that food waste has been considered as a matter of reduction and circumvention due to the adverse ecological outcomes associated with their disposal, it is also equally true that this food waste can act as a source of numerous value-added components [2,3] which can in turn, provide us with various health benefits. A befitting example in this context is the valorisation of fruit processing waste for the extraction of a valuable bioactive compound called pectin [4] which can be utilised to develop a prebiotic product. The current work focuses to discuss food waste valorisation to produce pectin and pectic oligosaccharides (POS) and how they can act as an efficient source for the realisation of prebiotic potential.

2. FOOD WASTE : A SERIOUS GLOBAL CONCERN

According to United Nations Food and Agriculture Organisation (FAO), about one-third of the world food production is wasted every year [3,6]. A finding issued by European Union in 2010 reports that almost 90 million tonnes of food waste is produced from the food manufacturing industry annually [7]. Producing more than 14 million tonnes of food waste in the year 2013, United Kingdom (UK) has undoubtedly become the kingdom with highest food waste generation in Europe [11, 12, 13]. Furthermore, food waste is anticipated to increase up to 126 million tonnes if effective steps are not undertaken [8]. In United States, approximately 61 million tonnes of food is wasted every year [10]. Food waste generation pace of 4 million tonnes per year has been acknowledged in Australia by Dee (2013) [10]. It has been reported that South Korea generates 6.24 million tonnes food waste per year, China generates 92.4 million tonnes per year and Japan accounts for 21 million tonnes of food production in the year 2010 [10].

According to Girottoet. al. (2015), in the present scenario, the definitions for Food Waste and Food Loss imbricate, therefore,the table below aims to give an insight on the yearly food wastage in some regions of the world.

Table 1: Annual Food Loss and Waste Per Region in 2007 (Adapted from Pleissner et. al., 2013)

	Food loss and waste (tonnes × 10^6)
Industrialised Asia*	357
South & Southeast Asia	275
Europe	205
Sub-Saharan Africa	127
Latin America	126
North America and Oceania	108
North Africa, Western & Central Asia	97
World	1,295

*Japan, China and Republic of Korea.

Wastage of food is wastage of an ocean of assets. According to The CSR Journal Report 2015 titled "Food Wastage in India A Serious Concern", when we waste food, we actually waste 25% of fresh water, acres of land and 300 million barrels of oil [5] and this list continues. As a matter of fact, according to the Agricultural Ministry, food produced worth of Rs.50,000crore is wasted every year in our country. We waste as much food as the whole of United Kingdom consumes [5].

The patterns of food waste generation from various segments of the world clearly indicate that no matter if it is a developed country or a developing one, both generate food waste to an enormously unacceptable extent [14], therefore, it has become a serious global concern.

3. FRUIT WASTE AS A SOURCE OF UNTAPPED POTENTIAL

Where there's muck, there's brass [15]. This holds equally true for fruit waste, which forms an appreciable portion of the total food waste generated. Also, since the early 1990s, as the focus shifts from waste treatment to waste prevention [16], there is certain section of waste which remains inevitable, therefore, now the emphasis is being laid on utilising this unavoidable class of waste [15] which can act as a potent source of multitude of bioactive compounds.

Here, as we focus on fruit waste, one notable thing is that the amount of disposed stuff in case of most of the fruit processing industries is huge [17,18]. For instance, in case of mango, 30% - 50% is waste, 40% - 50% is waste in case of pomegranate and in case of citrus fruits, 30% - 50% is thrown around like confetti [4]. Moreover, this fruit waste is loaded with enormous quantities of beneficial substances like carbohydrates, proteins, vitamins, minerals, oils and much more. Apples, citrus fruits, berries, olives, mango, pineapple, passion fruit – all produce large volumes of by-products [8, 19] which can be of vital use to mankind. Hence, it will be a wise move to treat fruit processing waste as a separate class of waste so that we can pay much more attention towards it as the major part of fruit and vegetable processing data for developing countries is found to scattered and deficient [20].

Amongst the entire long list of invaluable compounds which fruit waste can provide, pectin and pectic oligosaccharides (POS) hold a significant place. Our work aims to concentrate on the potential health benefits of these two substances.

4. PECTIN AND PECTIC OLIGOSACCHARIDES (POS)

4.1 Structure

Pectin can be termed as a family of polysaccharides that are abundant in galacturonic acid (Ga1A) [21]. Its construction is composed of "levelled" homogalacturonic (polygalacturonic acid) and bifurcating "hirsute" rhamnogalacturonic zones [22, 23]. Thus, it can be interpreted that the pectin structure is primarily made up of three elements :homogalacturonan (HGA), rhamnogalacturonan-I (RG-I) and rhamnogalacturonan-II (RG-II) [21,24]. Some sources also report the fourth component which is the xylogalacturonan (XG) [22, 25, 26, 27, 23]. All these pectin pieces are connected by either covalent or ionic bonding [28].

Homogalacturonan (HGA) : This component forms the major part of pectin as it constitutes more than 65 percent of pectin [22,23]. It is a collinear polymer consisting of identical units of (1- 4) – alpha – linked – D – galacturonic acid and is believed to encompass approximately 100-200 Ga1A remnants [21, 29, 30]. It is produced by the golgi bodies and is accumulated in the cell wall in such a way that its 70% - 80% of Ga1A residues are methyl-esterified at C-6 [24, 31]. If the methyl ester groups within the cell wall matrix are detached, HGA becomes available for cross-linking with calcium which results in the formation of supramolecular assemblies and gels. While other alterations also occur with HGA, methyl-esterification is the most predominant [21]. Ga1A remains in HGA can also be acetyl-esterified at C-2 and C-3.

Rhamnogalacturonan-I (RG-I) : is made up of alternate entities of Rhamnose and Ga1A [32]. Rhamnose is that small integrant of pectin pillar which is responsible for a sharp twist in the straight chain [22]. The main chain is interspersed with a number of side chains which can be either simple sugars or branched oligosaccharides. The extent of these side chains can range from single neutral glycosyl to polymeric side chains of various types like (1-5)-alpha-L-arabinans, (1-4)-beta-D-galactans, arabinogalactans-I, arabinogalactans-II [22]. The extent and the amount of HGA and RG-I varies with the raw material. For instance, the length of HGA series in sugar beet pulp is shorter than the ones in citrus and apple. On the contrary, RG-I is more profuse in sugar beet pulp than in citrus and apple [32].

Rhamnogalacturonan-II (RG-II) :has a complicated construction. It makes more than 10% of pectin [22, 24]. In spite of the close resemblance of its name with RG-I, RG-II does not structurally resemble RG-I [21]. It is in fact a clear section with HGA backbone [21, 22]. It is principally composed of galacturonic acid, rhamnose, galactose and unusual neutral sugars. It also includes a collection of side chains of rare sugar residues like apiose, aceric acid, 3-deoxy-lyxo-2-heptulosaric (DHA) and 3-deoxy-manno-2-octulosonic acid [22, 33].

Xylogalacturonan (XG) : It is a homogalacturonan (HGA) with substitution. It contains single unit of Beta-D-Xylp-(1-3) side chain [22, 27]. It is mainly found in those parts of plants which are responsible for reproduction and storage such as the cell walls of peas, soybeans, watermelons, onions, pears, apples, potatoes, pollen, pine and cotton seed [34, 22].

Pectic Oligosaccharides (POS) are produced by the depolymerisation of pectin [32]. These includes both

substituted and non-substituted oligosaccharides [32]. The most popular pectic oligosaccharides are arabinogalacto-oligosaccharides, arabinoxylo-oligosaccharides, arabino-oligosaccharides, galacto-oligosaccharides, oligo-galactouronides and rhamnogalacturonan-oligosaccharides [22, 32].

4.2 Sources

4.2.1 Apple pomace

Apple pomace is one of the principal sources from which commercial pectin is procured as it contains 10% - 15% pectin (dry basis). Kennedy et al. (1999) scrutinised the extremely changeable composition of apple pomace and the feasible schemes for its utilisation [35]. The study conducted by numerous research groups has led to the conclusion that pectin production is the most rational method of making use of apple pomace both from an economical and environmental aspect [35, 36].Furthermore, the factor that apple juice industry generates mountainous quantities of waste as about 75% of apple is used for juice making and the rest 25% is apple pomace [8, 37], pectin production from apple pomace is a good way to deploy this waste.Watt et al. (1999) also reports that apple pomace forms 25% - 35% of dry mass of an apple [40].

Although the two primary sources of commercial pectin are apple pomace and citrus peel, both of them yield different kinds of pectin [35, 38, 39]. Apple pectin is the preferred choice when it comes to bakery stuffings because its gel-forming ability is better than that of citrus pectin [35, 38, 39]. Citrus pectin, being light-coloured, is more adequate in case of confectionery jellies, but when it comes to orange marmalades, the hue imparted by liquid apple pectin is considered as an appreciable attribute [35, 38, 39].

Many innovative methods have and are being developed for the increased production of pectin from apple pomace. Research has shown that extrusion technique can be applied to apple pomace for the extraction of pectin which has both superior grade and yield [41]. Experiments were conducted on the influence of extraction conditions on the production and purity of apple pomace pectin precipitated but not washed by alcohol [42]. The results demonstrated that with the changing extraction conditions, pectin yield varied from 3.4% to 8.9% and the highest yields were attained with extreme extraction conditions at pH 1.5 [42]. Bleaching of apple pomace by alkaline peroxide treatment leads to depletion of polyphenols and deterioration of pectin [35, 43], so, in order to have a good pectin yield, bleaching by this operation must be avoided. A technique named enzymatic liquefaction with pectinases and cellulases boosts the release of phenolics but does not permit the retrieval of pectin [35, 44]. In 2001, a mechanism for the revival of both pectin and polyphenols was devised [35, 45]. The gel-forming ability of colour-improved pectin did not experience any change. For this reason, it can be concluded that separation of oxidised polyphenols with pure pectin characteristics which broadens the scope of its utility [35].

Apple remains act as a potent source of highly dividing RG and XG polysaccharides [22, 46]. If an oligomer of a particular chain length is needed, these polysaccharides can be further broken down [22]. Fucogalactoxyloglucan oligosaccharides can be derived from apple pomace through alkaline pretreatment [22, 40, 47].

4.2.2 Citrus waste

Citrus fruits are a whole different class of fruits which includes lemon, grapefruit, mandarin orange, bergamot orange, key lime, pomelo, citrus junos, bitter orange, citron, kaffir lime, tangelo, clementine, satsumamanadarin, meyer lemon, oroblanco, citrus leiocarpa, sweet lemon, finger lime, sudachi, persian lime, orangelo, amanatsu etc. They are regarded as the most vital fruits cultivated and consumed throughout the world [22, 48].

As compared to other fruits, the edible fraction of citrus fruits is less. As a result, sizeable volume of waste like seeds and peels are generated during juice and fruit processing operations [8]. For instance, 40 – 60 % of the orange weight, which mainly includes peel and segment pellicles, is discarded as waste from the orange juice processing industry [22, 49]. Matter which dissipates from the citrus juice-producing facility produces a whole array of invaluable substances like dried pulp and molasses, fiber-pectin, cold-pressed oils, essences, D-limonene, juice pulps and pulp wash, ethanol, pectin, seed oil, limonoids and flavonoids [35, 50,51, 52] and out of all the substances being obtained, pectin forms a significant percentage and acts as one of the principal sources of commercial pectin [38, 39]. A chief tropical crop, *citrus reticulata,* contains 10% cellulose, 4.28% hemicellulose, 0.56% lignin, 5.78% protein, 22.6% pectin and 3.23% ash, thus being a rich source of pectin [22, 53]. Lime peels can be used to recover fiber-pectins which are reported with high fiber value [52].

It should be ensured that when citrus waste like citrus peels are to be used for pectin production, they do not undergo de-esterification or degradation by mold attack. Mold attack makes the peels unfit for most of the final stage uses by releasing a diverse range of pectin de-esterifying (pectin methylesterase) and degrading (polygalacturonase, pectin lyase) enzymes [38]. In addition to this, citrus waste itself harbours consequential quantities of pectin methylesterase. Orange peel serves as a good example in this context as it is peculiarly rich in this enzyme [38]. Enzyme pectin methylesterase, unlike fungal pectin methylesterase, leads to chunks of de-esterified matter making the pectin more reactive to calcium than shown by its overall degree of esterification which can prove to be a drawback in various specific uses. For this reason, the practice of storing wet pomace or peel for more than a few hours, without any treatment, should be shunned. In fact, the transportation time involved in the

movement of raw material from the remote juice plant to the desired location also causes some loss in the final pectin produced. Citrus peels or pomace can either be used for pectin extraction soon after the juice extraction or they should be dried until further use. This makes it stable for months [38].

As far as production of pectic oligosaccharides (POS) is concerned, literature delineates the manufacture of POS from orange peel waste [54]. Notable quantities of oligosaccharides of DP > 2 has been reported in citrus juices which undergo natural fermentation [55]. The fabrication of numerous oligosaccharides during such fermentations is accelerated by the transfructosidase activity of invertases. POS produced through irradiation (10 kGy / h) from citrus pectin exhibited beneficial outcome on levels of serum triglyceride, total cholestrol and LDL-cholestrol in the blood of mice fed with high-cholestrol diets [56]. Green-labelled pectins and POS were extracted by an eco-friendly method using proteases and cellulases from plant by-products like citrus peel, chicory roots, cauliflower florets and leaves, endive and sugar beet pulps [57]. To be more precise, POS were obtained from the modified hairy regions of these plant by-products when remains collected after pectin extraction were enzymatically treated [57].

4.2.3 Sugar beet residue

Sugar beet residue has been noted as one of the sources of commercial pectin at the time of Second World War when it was utilised as an adjunct to apple pomace in England as well as in Germany [38]. Although it provides elevated quantities of pectin at a comparatively economical price, it cannot give a tough competition to the two main sources of pectin i.e. apple and citrus fruits [38, 39]. This is primarily because of its low molecular weight [38, 39, 58, 59, 60, 61] which acts as a barrier in its gel-forming capacity [38] and apparently high level of neutral sugars which sometimes results in the lowering of galacturonic acid content below the acceptable value [38]. Other pitfalls related to sugar beet pectin usage such as low degree of esterification and the existence of acetyl groups which obstruct gelation can still be dealt with the help of chemical changes but low molecular weight and high neutral sugar level still poses problems [38, 39]. Acetyl groups can be eliminated and the portion of ester groups can be increased using acidic methanol, however, doing this would lessen the already poor molecular weight [38].

Research analysis unveils one interesting feature of sugar beet pectin which is not found in apple and citrus pectin. It contains ferulic acid residue (0.6 % w/w) attached to the non-reducing residue of side chains, a feature similar to that seen in spinach pectin [58, 62, 39]. These ferulic acids have the ability to build cross-links on treatment with peroxidase and hydrogen peroxide to ultimately give rise to a thermally unreactive covalently cross-linked gel which if needed, can be dehydrated and rehydrated [38, 39, 58]. On this account, it can be inferred that sugar beet pectin might be put to use in applications which are altogether disparate from those of modern-day commercial pectins, including things that facilitate absorption and grasp multiple times their weight of water [38,39].

Substantial data has been reported by Babbar et al. (2016) about the production of POS from sugar beet pulp, a by-product of the sugar refining industry [22]. Sugar beet pulp polysaccharides comprise of about 22-24% cellulose, 30% hemicellulose, 15-25% pectin, 5.9% lignin and 3% ash [63]. The percentage of protein, fat and lignin is less. Research shows enough evidence for the successful production of POS from sugar beet pulp [64, 65]. Sugar beet arabinan of molecular weight ranging from 5700 – 10,000 Da and arabino-oligosaccharides have been isolated from sugar beet pulp [66]. Kuhnel et al. (2010) characterised branched arabino-oligosaccharides [having an alpha-(1,5)-linked backbone of L-arabinosyl residues] from sugar beet pulp produced by a mixture of arabinohydrolases [22, 67].

4.2.4 Olive pomace

It can be defined as a solid residue comprising mainly of skin, pulp, bits of kernel and some oil [8]. Its cell wall contains large amounts of pectic polysaccharides (39%), cellulose (30%), hemicellulosic polymers abundant in xylans and glucuronoxylans (14%) [22, 68, 69]. Olive oil processing release bulks of olive pomace as a by-product [22]. Largest production of olive oil takes place in Spain [22]. Not only olive oil processing in particular, olive food industry processing as a whole also produces olive pomace as one of its chief by-products [8,22].

A distinctive trait of pectic polysaccharides of olive pomace is the existence of arabinan. The degree of methyl esterification and acetylation in olive pomace is 48 and 11% respectively [70], indicating good gelling properties of pectin. Two properties namely, high arabinan and considerable galacturonic acid, makes it an ideal material for the extraction of POS [71, 72, 73]. Moreover, diverse conformations of neutral and acidic xylo-oligosaccharides as well as tetra-, tri- and di-galacturonic acid can be manufactured by hydrothermal treatment of olive pomace [72].

4.2.5 Others

Passion fruit processing also results in considerable amount of waste. This is because 75 % of the raw material is released as waste from these processing plants [35]. The peel of the fruit, which forms 90% of the waste, acts as a prime source of pectin [8, 78]. When nitric acid has been used for pectin extraction from passion fruit, the pectin produced was comparable to that of an industrial one as its chemical and rheological characteristics were quite similar to industrial pectin [8, 78].

Tomato is consumed in large quantities throughout the world. It accounts for 100 million tonnes of production in 144 countries [79]. As a result, it also produces sizeable volumes of waste. Tomato processing waste in particular is very rich in valuable compounds. When tomato processing by-products

were compared with unprocessed tomatoes in terms of availability of bioactive components, it was found that industrial tomatoes encompass consequential quantities of these beneficial substances [79]. In connection with this, oligo-galacturonic acid (DP 6-12) obtained from tomato processing plant after treatment by acid-hydrolysis proved to be an effective plant growth promoter [80].

Table 1 lists the sources of pectin along with their extraction method and yield.

4.3 Pectin extraction methods

4.3.1 Basic conventional method

As pectin is soluble in water but insoluble in alcohol or other organic solvents, acid is generally used to extract it from plant tissues. Mineral acid i.e. HCl is the commonly used acid. Then, it is precipitated using organic solvent like alcohol [81].

The traditional method of pectin extraction is termed as "hot acidic solution method" [81, 82]. Before carrying on with this actual process, pre-treatment is done in which the sample (e.g. apple pomace powder / orange peel powder / orange pomace powder) is blanched, dried and ground [75]. Then, it is to be made extractive-free. In order to make the sample free from lipids, sugars, polyphenols and all other extraneous matter, soxhlet extraction is conducted. Firstly, soxhlet extraction of the sample is done using hexane as a solvent. The aim of this step is the removal of wax, lipids and pigments from the powder. Secondly, ethanol is used as a solvent for the soxhlet extraction. This step removes proteins, free sugars and polyphenols. The end point in both the stages of soxhlet extraction is the colourless appearance of the siphon or of the trickling extract [75]. Once this preliminary procedure is accomplished, now the "hot acidic solution method" is followed [81, 82]. Here, the extractive-free powder is boiled with acidified water. As stated earlier, HCl is the most commonly preferred acid. During boiling, pH is maintained between 1.5 – 3.6 (pH value of 2 is commercially preferred) and temperature is kept around 70 – 90*C. Boiling time is generally greater than 1 hour [81, 82]. The extraction procedure of pectin is guided by the principles of mass transfer, so, factors such as boiling time, temperature, pH, viscosity play an important role. Therefore, they must be carefully monitored and regulated during extraction. After boiling, filtration takes place and then, the extracted pectin is precipitated using alcohol. Finally, drying is done at temperature ranging from 40 -45*C until constant weight is recorded [81, 82].

4.3.2 Commercial practice

For commercial purposes, hot mineral acid (HCl) is employed for pectin production. As mentioned earlier, pH used is 2 [38]. The treatment time of the raw material with acid depends on a number of factors: nature of raw material, kind of pectin desired, producers' knowledge and experience. After the hot acid treatment is over, separation of the pectin decoction from the solid remnant is a difficult task as the solids tend to lose their firmness at this stage and the viscosity of the liquid part keeps on increasing as the pectin concentration and molecular weight increases. Therefore, this stage demands a perfect balance between efficient extraction technique and running cost because extraction is enhanced with increased liquid content whereas running cost is managed by concentrating the extract.

The recovered extract is then filtered using a filter aid like kieselguhr. Particularly for liquid apple pectin, it is treated with carbon for colour elimination and with alpha-amylase to breakdown starch which pose a possibility of precipitation from the liquid product. Once the extract has been subjected to filtration, it is then concentrated using vacuum. This operation imparts effective concentration at low temperature and also keeps a check on pectin degradation. In every part of the process, it must be ensured that unreasonable holding durations at high temperatures are avoided.

The usual practice is that when pectin is obtained as a fibrous viscous mass, it is pressed and washed to get rid of the mother liquor and then dried and ground. A deviation from this usual precipitation procedure involves the use of colloidal aluminium hydroxide as pectin can be precipitated together with aluminium hydroxide. This method have been used many a times in the past and is till date being used in some fresh citrus peel processing plants. The fact that there is no need to concentrate the pectin extract in this process and also, certain unwanted substances are easily removed, makes it a preferred methodology. A pH of about 4 is attained by cooling the extract and adding it to a solution of aluminium salt and ammonia or sodium carbonate. Greenish-yellow coloured pectin flocculates and collects near the surface of the liquor which is separated by screening or flotation method. Following this, it is compressed to get rid of the remaining aqueous liquor. The collected yellow mass is then washed with alcohol and treated with acid in a series of steps in order to remove aluminium and finally, drying is done before partial neutralisation. Aluminium is not well-suited for the precipitation of pectin with high degree of esterification as precipitation is not very effective [83] and the general yield is usually less than that from the alcohol process.

Pectin extracted from different sources might exhibit different properties. So, if a product with a uniform set of characteristics is desired, it is better to go for a "blend" where product from various production lots is mixed together and diluted with sugar or dextrose to a standard performance [38].

The pectin production techniques discussed above provide "rapid set pectin" which have approximately 70% esterification or methylation value. If some other types are desired, these can be produced by the hydrolysis of ester groups. This is generally accomplished by using acid, either before or during an extended extraction, in the concentrated liquid, or in an alcoholic slurry before separation and drying. Through this process, an array of slower setting high methoxyl

and calcium reactive low methoxylpectins can be formed [38]. Instead of acid, alkali or ammonia can also be employed for hydrolysis of esters. When alkaline treatment is being used, one thing to be noted is that the temperature should be kept low, otherwise pectin undergoes degradation by beta-elimination reaction at neutral or alkaline pH [38]. If ammonia is used, "amidatedpectins" are formed as ester groups get converted into amide groups. These amidatedpectins are generally of the low methoxyl type.

Commercial pectin production generates lot of effluent which poses serious problems and in some cases, these problems have grown to an extent that it led to the closing down of the production plants. The major part of the effluent is the non-pectin organic part released due to the solubilisation of sugars and hemicelluloses present in the raw material. This organic material ends up in the aqueous stream from alcohol recovery, or in a more dilute form in the aqueous mother-liquor from aluminium precipitation [38]. It becomes a daunting task for the manufacturer to deal with the generated effluent as it involves economical and technical issues.

4.3.3 Enzyme-assisted extraction

The conventional method of pectin extraction is not environment-friendly primarily because of the use of acid and high temperature. It also causes deterioration of the equipment as the use of acids and high temperature leads to rapid corrosion [84]. In addition to this, with the increasing need to develop sustainable processes for food waste valorisation, enzymatic method of pectin extraction acts as a good alternative since it helps to conserve solvents and in fact, product obtained will also be of higher quality. It is one of the most widely-accepted processes when it comes to pectin extraction from agro-industrial by-products [81].

Enzymes are more selective in their approach than acids and this aspect provides a number of benefits during pectin extraction. For instance, if we use acid for pectin breakdown, it will accomplish the task by randomly breaking the glycoside bonds in order to yield short-chain oligosaccharides or monosaccharides whereas if enzymes are used for this purpose, they would be more targeted towards breaking some specific chemical bonds which will lead to the production of bigger pectin molecules. Another benefit of this selective nature of enzymes is that the quantity of solvent or chemical required as compared to acid hydrolysis is reduced or the other way round, the productivity for the same amount of solvent is increased [85]. The plant cell wall is a complex entity which comprises of an intertwisted mesh of polysaccharides namely cellulose, hemicellulose (e.g. xyloglucan) and pectin as well as proteins, showcasing certain interactions among them. As a matter of fact, the cellulose/ xyloglucan network is embedded inside the pectin lattice together with a protein web [82, 86, 87]. Certain cell-wall constituent degrading enzymes like cellulases, hemicellulases and proteases, which are very particular in their action and are also with least pectinolytic potential, have been used to support hydrolytic reactions in the concerned non-pectin plant cell wall components. The important thing to note here is that one must possess good understanding of the catalytic role of the chosen enzyme(s) as well as suitable circumstances for their use [82, 85, 88]. Degree of esterification (DE) of pectin extracted through enzymatic process is higher than that produced by acid hydrolysis. It is so because in addition to breaking of the glycoside linkages of cell walls, acid also hydrolyses the carboxyl bond from galacturonic acid which lowers DE. Enzymes, on the other hand, can yield pectin with a higher DE by avoiding these limitations [81].

In order to further highlight the contrast between the enzymatic and traditional acid extraction method, works of Wikiera, Mika and Grabacka (2015) and Panouille et al. (2006) can be referred to. Literature from the former source reports that enzymatically extracted pectin had higher molecular weight than conventionally extracted one, although their galacturonic acid contents were not very different from each other [90]. Panouille et al. (2006) studied enzyme-assisted as well as conventional method of pectin extraction from chicory and cauliflower. Enzymes used in the process were cellulases (celluclast) and proteases (Neutrase®). It was found that although pectin extracted from enzymatic technique had lower molecular weight than the one produced through the traditional method, its yield was higher.

Enzyme-assisted extraction works by following either of the two mechanisms: (1) pectin is degraded using enzymes and then it is separated (2) plant cell wall is disassembled using enzymes and then pectin is isolated [87]. Second mechanism is usual whereas the first one has also been reported by Zhao et al. (2015) and Zykinska et al. (2008) [57, 89]. In the work of Zhao et al. (2015), de-esterification of commercial high-methoxyl (HM) pectin into low-methoxyl (LM) by the application of high hydrostatic pressure (HHP) and enzyme pectin methyl esterase (PME) synergistically has been studied. Zykwinska et al. (2008) showed that LM pectin can be generated from an otherwise HM pectin source with the help of direct addition of PME along with cellulases and proteases into the enzymatic process.

Apart from discarding the use of high temperature and protecting the equipment from corrosion, enzyme-assisted process has a number of advantages. Its use results in a general decrease in the total time required for the extraction process because it helps us to avoid certain preliminary steps like extreme size reduction of the sample and making the sample free of extractives (e.g. sugars, lipids, pigments etc.). On the contrary, these steps cannot be circumvented in case of conventional extraction method [90]. One more reason for the quick enzymatic extraction process is presumably the augmented desorption achieved by means of enzymatic hydrolysis. Use of enzymes also eases out the load of legislative requirements to treat the effluents released from conventional method. Cost-effectiveness is also eventually attained by the industry using enzymatic method because less

use of solvents implies to less alcohol required for precipitation which ultimately leads to money-saving. The quality of pectin produced by this process is reported to be better in comparison to that obtained from other extraction techniques, provided optimum conditions are provided during the operation and other contributing factors are also suitably controlled [88, 91]. Besides this, the original structure and the native functions of the polysaccharides are conserved if enzymatic treatment is used for pectin extraction, as noticed in the case of seaweed hydrocolloids (agar, alginate and carrageenan) [92].

In spite of all the benefits offered by this method, its use is still very much limited to research labs. The first and the foremost issue is the cost of enzymes. When enzymes are used for pectin extraction in the lab, it is not a very expensive affair but at an industrial level, the cost factor takes a whole new level as the amount of raw material to be handled becomes large. Enzyme-controlled reactions require proper conditions such as temperature, nutrient availability etc. for good pectin yield. These conditions can be very well-monitored in labs but this may not be possible in big plants [85]. In fact, this conundrum in process control related to scaling up acts as a barrier in conducting many food industry processes [93].

4.3.4 Subcritical water extraction

Subcritical water is the liquid water which does not undergo change of state although it has been subjected to high pressure. This high pressure raises its temperature above the boiling point but phase change does not occur [81, 82]. The process of extraction which makes use of such water is known as Subcritical Water Extraction. It is also known as Pressurised Hot Water Extraction or Superheated Water Extraction [94]. This process has find its applications in various fields of study like food, pharmaceuticals [94] and environment where it may be recognised with different names [95,96] . For instance, the same process is addressed as Accelerated Solvent Extraction if any other subcritical solvent in place of water is being used [95, 96].

The whole system of Subcritical Water Extraction consists primarily of water supply connected to a high-pressure pump, an extraction column situated inside an oven, water cooling division attached to a pressure controller and the extract collector [81, 82]. As the pressure of the water (or any other subcritical solvent) increases, the target compound diffuses from the interior parts of the plant to the top of its surface, then it is conveyed to the bulk solvent and from here, this adsorbed substance is washed out with the solvent from the extraction column. Then, the separated compounds of interest are filtered, precipitated, dried, standardized and finally, its different properties are identified [81, 82].

Subcritical water exhibits a number of properties which act as an advantage in this treatment process. This liquid water at high temperature has good diffusive capacity, decreased viscous forces, low surface tension, increased vapour pressure and elevated mass transfer rates. Moreover, dielectric property and solubility characteristics are considerably changed when water is subjected to such raised temperatures [81, 82, 94, 98] and all this contributes to better extraction. For example, when water is subjected to temperature as high as 200*C, its dielectric constant reduces to 33 which was 79 at room temperature (25*C) and with such reduced value of dielectric constant, it becomes feasible to draw out both ionic and non-ionic target compounds from the substrate [97, 99, 100]. In addition to all the advantages offered by the use of subcritical water, this extraction technique provides extracts of higher grade at low cost as compared to other processes. The quantity of solvents used is also less, thus contributing to cost-saving and the extraction process is quick [94, 97]. The fact that this treatment makes use of solvents like subcritical water which are generally recognised as safe (GRAS) makes it a suitable choice for extraction of medicinal and food grade substances like pectin [82].

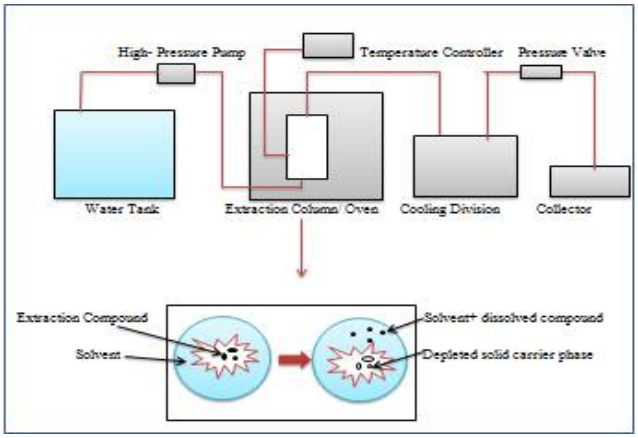

Figure 1: Schematic Diagram of Subcritical Water Extraction (Adapted from Ueno et al. (2008) and Zakaria and Kamal (2015))

It can be concluded through literature that low values of solid/liquid (S/L) ratio produce optimum results. When Wang and Lu (2014) used apple pomace to extract pectin through subcritical water extraction, the S/L ratios ranged between 1:4 and 1:14. The best yield was obtained with 1:14 S/L ratio because it has been reported that low S/L ratios are much more suitable for the occurrence of mass transfer at the time of extraction due to lower viscosity of pectin. The temperature and time range used was 140 to 160*C and 5 to 15 mins. respectively. The optimum temperature was found to be 140*C and 5 minutes was reported as the ideal time. 16.75% was the maximum yield obtained in this work [81,101]. In another research work conducted by Wang and others (2014), subcritical water extraction was used to extract pectin from apple pomace as well as citrus pomace. In case of apple pomace, the temperatures employed were 130, 150 and 170*C. The extraction time was kept 5 mins and the S/L ratio used was 1:30. Particles of 100-mesh sieve size were added to distilled water, mixed well and autoclaved. 16.68% at 150*C was reported as the maximum yield [81].

As far as the drawbacks of this process are concerned, pectin degradation might occur if process parameters like temperature, time, S/L ratio are not adequately controlled [102]. The other factor which normally acts as the primary hindrance is the cost involved in implementing this technique [82].

4.3.5 Ultrasound–assisted extraction

As the name indicates, this extraction process makes use of ultrasonic waves. The frequency of these waves is greater than the upper limit of the audible range of human ear (1-16 kHz) and is reported with frequencies from 20 kHz. Unlike electromagnetic waves, these sound waves require a medium to disseminate. The medium can be solid, liquid or gas. As the waves propagate through the medium, its molecules undergo a sequence of expansion and compression which in turn, leads to formation of cavities (cavitation). These cavities form the very foundation of ultrasound-assisted extraction [103]. Expansion process makes the molecules go away from each other whereas compression pulls them back together and tries to keep them intact and in case of a liquid medium, this expansion process leads to the formation of bubbles which subsequently grow bigger and ultimately collapse as the negative pressure applied becomes greater than the liquid's local tensile strength. This whole phenomenon by which bubbles form, get bigger and finally exhaust is termed as cavitation. It creates pressure up to 1000 atm. and temperatures go as high as 5000*C for a very short span of time (approx. 400 µs) thus facilitating greater extraction of substances from plant tissues [98,103].

Utrasound-assisted extraction set up can be accessed either through bath or probe. For pectin extraction experiments in particular, usage of probe is more common as far as lab scale work is concerned. However, in general, bath is more popular but it suffers from two major disadvantages: (1) non-uniform dispersal of ultrasound energy (2)dissipation of energy provided to bath as the system experiences dwindling of power with time. As a whole, using ultrasonic probes is a better option as they result into effective cavitation because their energy is more focused on a certain sample region more effectively as compared to bath units [103, 104].

Plant attributes as well as the ultrasound system control parameters are the two primary factors which determine the proper working of the ultrasound-assisted extraction method. Plant attributes mainly comprise of its moisture percentage, particle size and the solvent used. Ultrasound system control parameters, on the other hand, encompass temperature and pressure conditions, frequency, sonication time. In addition to all these parameters, solvent-biomass ratio also plays an important role as the increase in solid matter results in weakening of ultrasound intensity [105].

As people have started to comprehend the principle behind this extraction technique in a better way, the studies in this field for pectin extraction are increasing. For instance, when three different pectin extraction methods, namely conventional method, microwave-assisted and ultrasound-assisted extraction methods, were compared in case of grapefruit peel, it was reported that intermittent sonication in water bath is better than continuous sonication as it promised good productivity. Sonication time and bath temperatures were observed to be the main parameters affecting ultrasound-assisted extraction, with most favourable values, 25 mins. and 70*C respectively. In spite of this, the yield (27.81% (w/w) db) by microwave-assisted extraction was higher than the ultrasound-assisted extraction which was acknowledged with a yield of 17.92% (w/w) db [106].

4.3.6 Microwave-assisted extraction

Microwave is a blend of two fields: electric field and magnetic field. These two fields are at 90* to each other. The frequency of these waves range from 300 MHz to 300 GHz [107]. Unlike conventional heating method which comprises of phenomenon like conduction and/or convection, microwave heating method is based on the generation of heat from vibrating electromagnetic field [81]. When polar molecules vibrate at a high frequency, heat is produced within the material which leads to greater heat transfer throughout the material. It is because of all these factors that microwave-assisted extraction is anticipated to enhance the extraction of bioactive molecules such as pectin from deep within the plant tissues. For instance, when microwave-assisted pectin extraction from orange peels was compared with the conventional one, it was concluded that microwave method gave higher pectin yield in less time [108].

Numerous studies of pectin extraction by microwave-assisted extraction have been acknowledged in case of apple pomace. Before using apple pomace for pectin extraction, microwave heating has been used for their pre-treatment [109]. The degree of esterification and gel strength were observed to be greater than the control sample after this pre-treatment. This affirmative outcome of employing microwave heating on the productivity and quality of pectin can be attributed to two main elements (1) partial breakdown of plant tissue and hydrolysis of protopectin (2) fast deactivation of pectolytic enzymes in the raw matter [81]. Wang et al. (2007) implemented microwave heating for pectin extraction from apple pomace. 2 g of apple pomace powder was added to HClsolution, mixture was agitated and put to microwave at 2450 MHz under stirring. All the main process parameters namely extraction time, pH, solid/liquid ratio and microwave power were optimised using response surface methodology. The most befitting conditions were reported to be : 20.8 minutes as the extraction time, 1.01 pH, 0.069 solid/liquid ratio, 499.4 W microwave power and the maximum yield was as high as 23% [110].

One main point which makes this extraction technique most appealing is the fact that in all its ways, it can be attributed as a green technology since it has less solvent needs coupled with less extraction time. Also, there are some more specific factors

hidden behind these main factors which have been established as an advantage in other microwave-related uses of food industry. First one is that microwave-assisted extraction permits uniform temperature distribution in the medium thus preventing the formation of a temperature slope. Second one is that the paraphernalia size is noticeably reduced with the use of this extraction method. As compared to conventionally extracted pectin, microwave-extracted pectin is found to be better in terms of quality as well as quantity [111].

The disadvantage suffered by this technique is that it still can cause equipment wear and tear as well as corrosion issues because irrespective of the lower acid solvent quantity employed in this method, we still cannot completely avoid its use.

3.4 Prebiotic potential of Pectic Oligosaccharides (POS)

Literature review indicates that POS act as a potential candidate for next-generation prebiotics.

Martin et al. (2002) compared the in-vitro bifidogenic characteristics of pectins and pectic-oligosaccharides. There were four study samples:two were of pectin- HMP and LMP whereas the other two were their corresponding oligosaccharides – POS I and POS II. Through this study, it was concluded that POS serve as a better prebiotic candidate than pectin. In order to calculate the fermentation ability, growth of four microorganisms, namely, Bifidobacteria, Lactic Acid Bacteria (LAB), Bacteroides and Clostridia was chosen as the benchmark and accordingly, Prebiotic Index (PI) was calculated. When HMP, LMP, POS I and POS II were utilised as carbon source in the mentioned bacterial cultures, it was found that Pectin fermentation exhibited zero selectivity towards bifidobacteria whereas in case of both POS I and POS II, bifidobacteria population increased and the Bacteroides as well as Clostridia population remained constant. Hence, it was observed that POS prove to have better prebiotic power than pectins and the PI score for each carbon source reflected the same conclusion. PI score of pectins dropped and that of POS increased as the fermentation proceeded [112].

POS-rich abstract obtained through enzymatic extraction from Bergamot peel has the capability to act as a good source of prebiotics by selectively increasing the number of bifidobacteria, lactobacilli and eubacteria and declining the population of clostridia [113]. When the prebiotic performance of the Bergamot peel POS was compared to that of FOS, it was reported that PI score after 10 h incubation were higher for former thus ascertaining better prebiotic potential. Another point to be noted here is that the existence of bergamot extract promoted the flourishing of both bifidobacteria and lactobacilli whereas as per the Martin et al. (2002) study, only bifidobacteria growth was improved in case of orange peel substrates.

It has also been reported in literature that orange peel POS has the capability to produce a more prolonged prebiotic fermentation [114] than FOS because when in-vitro testing of the former was being conducted, it was noted that it increased the bifidobacterial population later in the fermentation process than did FOS. In addition to this, POS also provided an advantage of butyrate production by enhancing the numbers of Eubacteriumrectale. These group of bacteria are known to produce excessive quantities of butyrate which is very good for health. Butyrate prevents ulcerative colitis [115] as well as colon cancer [116, 117].

When a number of commercial prebiotic oligosaccharides viz. xylo-oligosaccharides (XOS), galacto-oligosaccharides (GOS), fructo-oligosaccharides (FOS), isomalto-oligosaccharides (IMO) and inulin were compared in terms of their in-vitro fermentation properties, it was concluded that IMO and GOS, which is a type of POS, are effective prebiotics as they resulted into expansion of bifidobacteria and lactate population as well as generated lowest gas volumes. The study also noted that oligosaccharides which contained galactose like GOS are more effectual than oligosaccharides like FOS and inulin which are fructose-based. This statement has been affirmed on the basis of growing numbers of bifidobacteria, increased lactate production and low gas generation [118].

According to a study conducted by Chen et al. (2013), POS developed by a process called Dynamic High-Pressure Microfluidisation (DHPM) portrayed characteristics very much similar to those of most extensively studied prebiotic i.e. FOS as far as growth of bacteria (bifido, lacto, bacteroides, clostridia) and production of short-chain fatty acids is concerned [119]. This clearly indicates that POS prepared by DHPM has a potential to be an effective prebiotic. If compared with pectin, POS increased the Bifidobacteria and Lactobacilli population and also resulted into greater concentration of acetic, lactic and propionic acid than their parent pectin. These oligosaccharides lowered the number of Bacteroides and Clostridia while their parent pectin augmented them.

5. CONCLUSION

Considering the predominant amounts of pectin present in fruits and vegetables, Pectin and pectic oligosaccharides (POS) production is certainly one of the most effective ways of food waste utilisation. Moreover, the fact that POS has great potential to act as a prebiotic source makes it an interesting compound to be studied further in depth because as the review indicates, the prebiotic study of POS is still at a nascent stage.

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