EXTRACTION OF PECTIN FROM SUGAR BEET PULP BY HIGH HYDROSTATIC PRESSURE AND INVESTIGATION OF EXTRACTION EFFICIENCY AND EXTRACT CHARACTERISTICS

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EXTRACTION OF PECTIN FROM SUGAR BEET PULP BY HIGH HYDROSTATIC PRESSURE AND INVESTIGATION OF EXTRACTION EFFICIENCY AND EXTRACT CHARACTERISTICS

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ABSTRACT

EXTRACTION OF PECTIN FROM SUGAR BEET PULP BY HIGH HYDROSTATIC PRESSURE AND INVESTIGATION OF EXTRACTION EFFICIENCY AND EXTRACT CHARACTERISTICS

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Food industry produces huge amount of food waste after food processing. The food wastes could include significant amount of functional ingredients that have valorization potential. The techniques utilized to obtain functional compounds from food wastes are named as valorization. One of the important wastes of food industry is sugar beet pulp which is the waste of sugar processing. Utilizing this pulp for various applications has been very common recently. In this study, sugar beet pulp pectin was extracted using conventional extraction and high hydrostatic pressure (HHP) assisted extraction. HHP was applied prior to extraction to ease detachment of pectin from cell wall. Different pressures (250, 350, 450 MPa) at 40°C for 5 min, two different extraction temperature (80°C, 90°C) and three different time (3, 4, 5 h) combinations were applied. Moreover, conventional extraction (CE) was compared with the results of HHP assisted extraction. Extraction yield, degree of esterification (DE), galacturonic acid content (Gal-A), rheological properties and water holding capacity (WHC) of pectin solutions were investigated. Obtained pectin was also investigated with FTIR Spectroscopy for structural elucidation. In addition, water holding capacity experiments were conducted by using Time Domain NMR Relaxometry. Extraction yield was almost doubled at HHP assisted extraction as

12.23±0.13% regarding 6.43±0.07% CE yield. HHP assisted extraction showed increasing DE values at prolonged extraction times but overall change was between 32-38% which was low enough to not reflect in viscosities of extracted pectin solutions. Change in viscosities were mostly insignificant (p>0.05). Gal-A decreased with increasing pressure, but it was still in safe limit regarding 60-65% Gal-A requirement of FAO. WHC was held insignificantly changed (p>0.05) at HHP assisted extraction by adjusting pressure considering the same temperature-time of CE. The results suggest that HHP assisted extraction is highly effective on increasing yield and modifying structural and functional properties of extracted pectin.

Keywords: Pectin, Sugar beet pulp pectin, Degree of esterification, Galacturonic acid, High Hydrostatic Pressure

ÖZ

YÜKSEK HİDROSTATİK BASINÇ İLE ŞEKER PANCARI POSASINDA PEKTİN ELDE EDİLMESİ VE ÖZÜTLEME VERİMİ İLE ÖZÜTLENEN PEKTİN ÖZELLİKLERİNİN İNCELENMESİ

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Gıda endüstrisi, gıda işleme süreçleri sonucunda büyük miktarda gıda atığı üretmektedir. Fonksiyonel besin öğeleri içerebilen bu gıda atıklarının değerlendirilme potansiyelleri yüksektir. Gıda atıklarından fonksiyonel bileşenlerin elde edilmesinde kullanılan teknikler, atık değerlendirme olarak adlandırılmaktadır. Gıda endüstrisinin en önemli atıklarından biri, şeker üretim süreçlerinin atığı olan şeker pancarı posasıdır. Bu çalışmada, konvansiyonel özütleme ve yüksek hidrostatik basınç (YHB) destekli özütleme yöntemleri kullanılarak şeker pancarı posasından pektin özütlenmiştir. YHB, pektinin hücre duvarından ayrılmasını ve özütleme verimine katkıda bulunma derecesini kolaylaştırmak için özütleme işleminden önce kullanılmıştır. Numunelerde farklı basınç (250, 350, 450 MPa) 40°C sıcaklıkta 5 dakika süre ile, iki farklı özütleme sıcaklığı (80°C, 90°C) ve üç farklı özütleme süresi (3, 4, 5 h) kombinasyonları test edilmiştir. Ayrıca, YHB destekli özütleme sonuçları, konvansiyonel özütleme yöntemiyle karşılaştırılmıştır. Özütleme verimi, özütlenen pektinlerin esterleşme dereceleri (DE), galakturonik asit içerikleri (Gal-A), pektin çözeltilerinin reolojik özellikleri ve su tutma kapasiteleri incelenmiştir. Elde edilen pektinlerin yapısal özellikleri, FTIR Spektroskopisi ile incelenmiştir. Ek olarak, su tutma kapasitesi, zaman alanlı NMR Relaksometresi kullanılarak ölçümlenmiştir. Konvansiyonel

özütlemede % 6.43 ± 0.07 olan veriminin, YHB destekli özütlemede % 12.23 ± 0.13 bulunarak neredeyse iki katına çıkmıştır. YHB destekli özütleme ile elde edilen pektin örneklerinin, uzayan özütleme sürelerinde esterleşme derecelerinde artış görülmüştür fakat tüm DE sonuçlarının % 32-38 aralığında olması, esterleşme derecesindeki düşüşün viskoziteye yansıyamayacak kadar düşük olduğunu göstermiştir. Viskozitelerdeki fark, pektin örneklerinin çoğunluğunda önemsiz derecededir (p>0.05). Artan basınç ile Gal-A içeriğinde düşüş bulunmuştur; fakat bu düşüşe rağmen Gal-A içeriği FAO tarafından belirlenen %60-65 limitinin altına düşmemiştir. Su tutma kapasitesi, YHB destekli yöntemde kullanılan basıncın dengelenmesi ile konvansiyonel özütlemede kullanılan aynı sıcaklık-süre kombinasyonları kullanılarak elde edilen verilerle benzerlik göstermektedir (p>0.05). Sonuçlar, YHB destekli özütlemenin, özütleme verimi üzerinde oldukça etkili olduğunu, bu yöntemle elde edilen pektinlerin yapısal ve fonksiyonel özelliklerinin modifiye edilebildiğini göstermektedir.

Anahtar Kelimeler: Pektin, Şeker Pancarı Posası Pektini, Esterleşme Derecesi, Galakturonik Asit, Yüksek Hidrostatik Basınç

To my family and love of my life Suat Altın

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INTRODUCTION

1.1. Valorization

The food industry produces a huge amount of food waste and by products after food processing which is approximated as 1.6 billion tons by FAO (2013). Regarding European Union only, this waste is expected to reach 1.26 billion tons/year at unless preventive actions (Black & Michalopoulos, 2017). According to FAO report the loss in harvesting and processing products is higher than 30%. The most waste producing industries are beverage, dairy and fruit and vegetables industry (Arshadi et al., 2016). The loss in fruit and vegetable industry divides into two as pre-harvest and post-harvest loss; but post-harvest loss that occur mostly in processing stage creates the highest loss (Akgün et al., 2019; Tatlıdil et al., 2013). These losses may cause wasteful consumption of food sources and scarcity while it is also an important item of environmental issues. Regarding these impacts, specialists qualified for food wastes proposed options as action steps for limiting food loss by waste (Lovrencic et al., 2017). These options were ordered for implementation of organizations all over the world where disposal was identified as last and least preferred option. Until getting through to disposal step, suggested action recommend utilizing wastes by dividing them into groups such as fruit skin, seed and pulp. By conducting the mentioned options, it is aimed to reach a significant reduction in food waste and this issue is one of the subjects of United Nations Sustainable Development Goals (2015). The food wastes could include significant amount of functional ingredients that could have valorization potential. There are so many techniques in literature that shows to obtain functional compounds from food wastes and all of these separation processes of valuable compounds are named as *valorization*. Recovery of phenolic compounds and anthocyanin from grape pomace (Barba et al., 2015), extraction of proteins, phenolics

and isothiocyanates from papaya seeds (Parnikov et al., 2015) and extraction of polysaccharides from mushrooms (Rosello-Soto et al., 2016) are just some examples to valorization of high-added value compounds.

Isolation of bioactive compounds from food wastes is gaining popularity with developing technology and employment of novel technologies in industry. Conventional extraction methods are followed by novel methods such as enzyme assisted methods, supercritical fluids, high hydrostatic pressure, ultrasonic waves and microwaves (Sagar et al., 2018). Coloring material isolation from fruit and vegetable wastes like lycopene of tomato has been studied by Baysal et al. (2000) by employing supercritical carbon dioxide. After that, Nobre et al. (2012) revealed that recovery of coloring materials by using supercritical ethane gives higher extraction efficiencies at shorter processing times. Another research was carried out by Alexandre et al. (2017) with application of high pressure as an assisted method to extraction for isolating phenolics, flavonoids and tannins of by-product of fermented fig.

Pectin is one of the valuable compounds lost in waste stream during food processing, especially in fruit and vegetable processing. The waste stream of plant material processing is used as animal feed, fertilizer or disposed where it is significant source of pectin (Christiaens et al., 2015).

1.2. General View of Pectin

1.2.1. Structure of Pectin

History and the name of pectin come from study of a scientist whose name is Henri Bracannot. By looking at Greek word “pektikos” which means coagulated compound, the name of pectic acid is nominalized. Henri Bracannot isolated a compound from

vegetables in 1825 and firstly described the compound which is named as pectin (Muzzarelli, 2012).

Pectin is a heterogeneous complex macromolecule found in cell wall of land growing plant, more particularly fruit and vegetables. This polysaccharide consists of fewest 17 different monosaccharides and D-Galacturonic acid (Gal-A) is the backbone and predominant unit of pectin. Remaining structure includes significant amounts of L-Rhamnose, D-Galactose, D-Arabinose and various amounts of other different monosaccharides (Vincken et al., 2003). The mentioned different monosaccharides and various amounts of them gives pectin heterogeneity (Naqash et al., 2017).

Chemical structure of pectin is highly dependent on its source and where it is found in the plant. However, common characteristic of all pectin molecules is to have a (1-4) linked α-D-Galacturonic acid units as backbone and neutral sugars bound to this backbone as side chains (Mohnen, 2008). Pectin can be divided into two structures; linear and hairy region. Linear region, that is to say backbone, is represented by linearly located units of α-(1-4)-D-Galacturonic acids bond to each other namely homogalacturonan (HG). This linear homopolymer is partially esterified by methyl groups at sixth carboxyl group and form the 60-65 % of the pectin molecule. Hairy regions are represented by branched structures including rhamnogalacturonans (RG) where RG-I is the main branch structure and RG-II is substituted HGs. The structure of hairy part is more complex with respect to HG and varies with substitution of many different oligosaccharides. The variation of RG-I and RG-II creates functional specializations of pectin molecules. RG-I is the backbone of branches which forms the 20-35 % of pectin molecules. It forms from repeating disaccharide bond of [→4)-α-D-Galacturonic acid-α-(1,2)-L-Rhamnose-(1→] (Mohnen, 2008). RG-II is the most complex part of the pectin molecule and forms approximately the 10 % of pectin. It

consists of α-(1-4)-D-GalA residues decked with many different sugars and linkages. The structure of pectin molecule can be seen in following Figure 1.1.

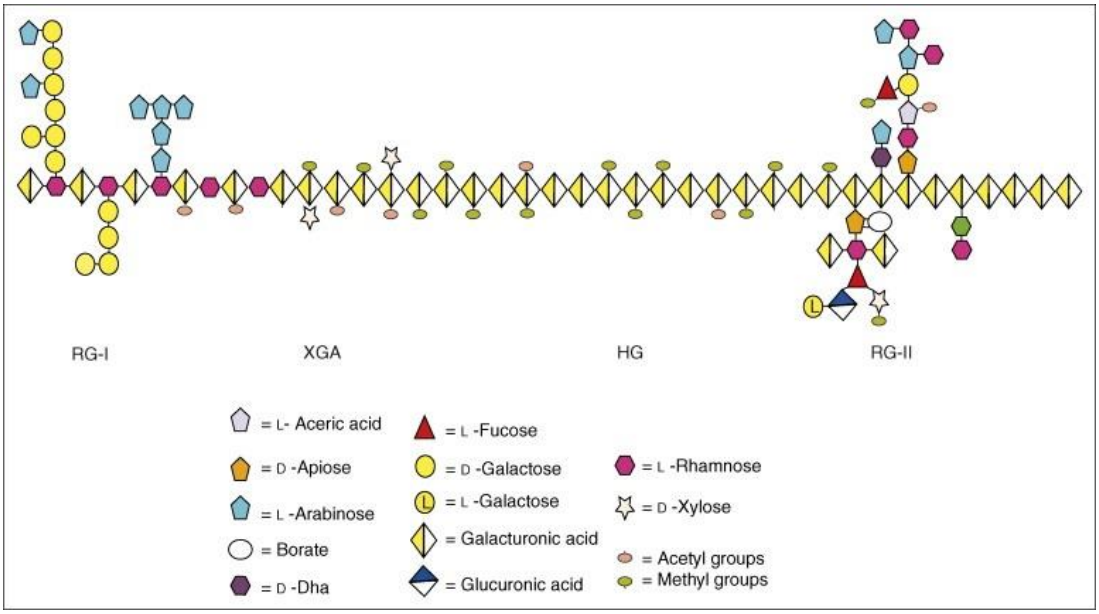

Figure 1. 1. Structure of pectin molecule (Mohnen, 2008)

1.2.2. Functions and Properties of Pectin

The name of pectin comes from a Greek word “pektos” which means viscous, firm, weighty and hard. Pectin was characterized in this way because it has high molecular weight and ability to closely pack or connect with polymers found in cell wall. The various structural characteristics of pectin make the molecule highly useful for many food and pharmaceutical applications.

Although pectin is a high molecular weight compound, pectin derivatives can be obtained by modifying the structure chemically or enzymatically and form low molecular weight pectin (Adetunji et al., 2017). Modified low molecular weight pectin is reputed to have positive effects on health such as anti-tumor activities against colon

cancer, effect on intestine against persistent diarrhea, enhance cardiovascular system by cholesterol lowering effect and reduce insulin and some polypeptides known as gastric inhibitory polypeptides (Almeida et al., 2015; Brown et al., 1999; Hasselwander, 2008; Maxwell et al., 2012; Rabbani et al., 2004).

The most common use of pectin finds place in texturizing applications in food systems. It is used as gelling agent, thickener, and stabilizer. The primary use of pectin in food industry is to use it as gelling agent for jams and jelly production, fruit juices, bakeries and confectionary products. Gel formation is defined as formation of three-dimensional networks that traps water and solute in it and enhance the rigidity of food system (Bhattacharya & Saha, 2010). In that way, the resistance of food system to flow is developed. Pectin gel formation occurs when crystalline network is formed by crosslinked HG units. Both solutes and water is trapped in between crosslinks where the trapping ability points to gelling ability and highly depends on pectin type, esterification degree of pectin, presence of calcium, pH and sugar content of solution (Willats et al., 2006). If esterification degree of pectin is high, crosslinks between HGs are formed by both hydrogen bonds and hydrophobic interactions of methoxyl groups in esterified parts. This crosslink formation goes along with high sugar concentration and low pH of solution. If esterification degree of pectin is low, presence of calcium promotes the crosslink formation.

Thickening ability is another functionality of pectin that makes pectin valuable for food industry and helps industry to modify rheological properties of food systems. These properties indicate flow behavior, in other words viscosity and texture. Viscosity and texture of food product influence the sensorial properties so their modification with food additives gains importance. Regarding the pectin being a heterogeneous long chain polysaccharide with its hydrophilic property, it forms viscous dispersions in water. So, pectin is said to be a hydrocolloid or hydrophilic

colloids. In the environment includes high number of hydroxyl groups, affinity of pectin to bind water molecules increases significantly. The viscosity of pectin solution originates from the disordered conformation of molecules in dispersion (Bhattacharya & Saha, 2010). In very dilute solutions, movement of molecules is free and easy which makes the solution less viscous. When concentration of pectin increases in solution, pectin molecules starts to contact with each other, and molecules cannot move freely. The shift from free movement for pectin molecules to restricted movement is the mechanism of thickening. This process is dependent on intermolecular interactions of pectin, concentration of pectin in solution and molecular weight of pectin (Sworn, 2004).

Stabilizing ability of pectin generates another usage area for it especially for acidified milk drinks. The mechanism behind the stabilization of system by the help of pectin is similar with the mechanism of casein micelles repulse each other at pH 6.7 and prevent flocculation (Tromp et al., 2004). Normally, casein micelles are in the suspended form thanks to the steric repulsion between micelle structures. However, while processing the milk to produce yoghurt or buttermilk the acidification step decreases the pH approximately around to 4 where stabilization mechanism of casein micelles does not work anymore. This is thought to be related with the conformational extension of κ-casein chains at pH 4. So, pectin is used for stabilization of the system. The stabilization mechanism of pectin to acidified milk drinks starts with electrostatic interaction between pectin molecule and casein micelles. After that, adsorption of pectin on casein micelles occur on charged parts of the pectin. The uncharged parts forms extended loops in the solution and these loops create steric repulsion just like κ-casein chains create at 6.7 pH. As a result, the acidified milk drink becomes stabilized even it has low pH. Following Figure 1.2 shows pectin absorption of casein micelles at different pH values.

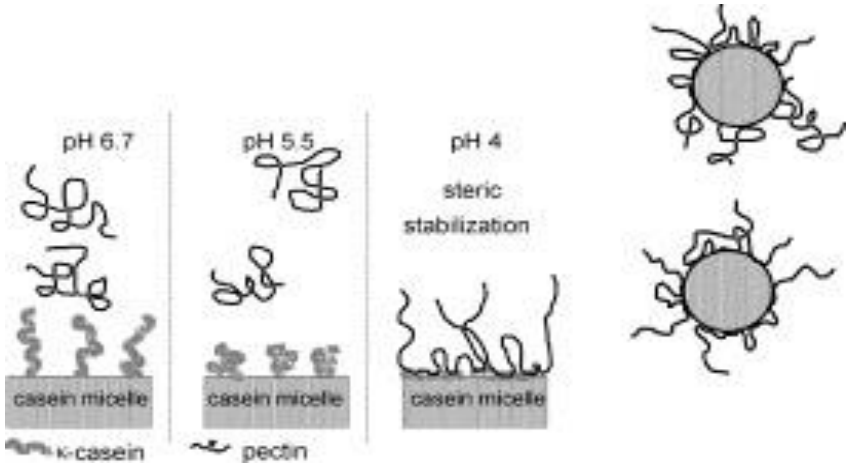

Figure 1. 2. Casein and pectin replacement at low pH. Pectin absorption of casein micelles (Tromp et.al., 2004)

Pectin increasingly gains acceptance as an emulsifier or emulsion stabilizer. The conditions effecting this property of pectin and usage of it as an effective emulsifier requires deep understanding; however, explanation of emulsion stabilization property of pectin has been rarely studied and found in literature. It was found that pectin extracts include low amount protein which changes from trace amounts to 5 % based upon extraction conditions and plant as pectin source (Akhtar et al., 2002; Sphigelman et al., 2015; Mesbahi et al., 2005). The existence of protein brings pectin polymer surface activity at water-oil interface (Ngouemazong et al., 2015). Consequently, it becomes possible for pectin to form or stabilize fine oil droplets during or after emulsification (Akhtar et al., 2002). The basic features of emulsifiers are the ability to significantly decrease interfacial forces at water-oil droplet interface by hydrophobic part strongly adsorbed by interface and hydrophilic part extending in water phase, and convenience of molecule to provide a stabilized structure to prevent flocculation (Dickinson, 1998). Due to the dominating hydrophilic property of pectin that is combining with surface activity of its hydrophobic protein, methyl and acetyl groups; it shows emulsifier characteristics and provide a steric repulsion to prevent droplet flocculation (Ngouemazong et al., 2015).

1.2.3. Pectins from Different Plant Sources

Pectins from different plant sources show significant changes in terms of existence percent and composition. The change in composition of this heterogeneous complex macromolecule causes variations in properties of molecule. Increasing acceptance for pectin as functional ingredient in food systems create a huge global demand and so finding new source to obtain pectin in the most efficient way become an important topic. There are studies that have discussed pectin content of diverse fruits and vegetables itself and their food wastes, such as banana, strawberry, pea, tomato, pumpkin, parsley, cauliflower, apple, apple pomace, apple peel, citrus peel, sugar beet pulp, pumpkin kernel cake, grape pomace, olive pomace, and the list goes on (McKnee & Latner, 2000; Müller-Maatsch et al., 2016). The results of studies show that almost all of these streams are valuable sources including pectin where there is remarkable diversity in structure, composition and properties of pectin. Moreover, factors such as growing conditions of plant, harvesting time, storage duration and imposed upon treatment also have great effect on pectin that will be extracted from the plant source.

In literature, the plant source contains the highest total pectic polysaccharides is citrus peels, hence citrus peels are the most common sources for pectin extraction (Müller-Maatsch et al., 2016). The highest GalA content has been found in apple pomace which is secondly most preferred pectin source (May, 1990). The highest esterification degrees have been recorded in apple, apple pomace, tomato, and berries (Hilz et al., 2005; Seymour et al., 1990; Müller-Maatsch et al., 2016). Between all plant sources, sugar beet pulp pectin was found to have the best emulsifying properties because of the higher protein content and so better surface activity of it (Ma et al., 2013; Huang et al., 2017).

1.2.4. Types of Pectin

One of the most important aspects that specify the physiochemical properties of pectin is its structure. Pectin structure changes with esterification of carboxyl groups on galacturonic acid units with methanol (Hosseini et al., 2016). The percentage of galacturonic acid units esterified in total amount of galacturonic acid in pectin gives the degree of esterification or degree of methoxylation (Flutto, 2003). The degree of esterification is substantial in the matter of identifying functional property and application area of pectin. Starting from this point, pectin molecules can be categorized into two groups as high methoxyl pectin (HMP) and low methoxyl pectin (LMP). The pectin molecules having esterification degree less than 50 % are named as LMP while the pectins its esterification degree higher than 50 % are named as HMP (Adetunji, 2017). The application areas of these two forms are different. HMP has the ability to form strong gels in low pH and high sugar content conditions. However, LMP is able to form gels regardless of pH adjustment and it does not require high sugar concentration, it works in a wide pH range between 2 to 6 and even in very low sugar by the help of divalent cations like calcium ion (Fishman et al., 2007). Regarding this, HMPs are used in food industry as food additives for the gelling, thickening, stabilizing and emulsifying functions where LMPS are used as fat replacers especially for ice cream or fruit yoghurt products.

HMPs are also divided into two groups in accordance with rapidity to form gel structure as rapid set pectins and slow set pectins. Rapid set pectins are preferred if the gel will be formed at high temperatures so it is all-purpose for jams and preserves (Smith, 2003). Owing to forming gel structure is speedy; it is prevented for the fruits to move through surface before gel is formed. Besides all these, pectin is commercialized by giving its grade where the most common commercially available pectins are 150 grade and 100 grade pectins. Its grade indicates how many times of

sugar is required to form exactly the same structured gel formed by using a unit weight of pectin (Smith, 2003).

1.3. Industrial Application of Pectin

Industrial use of pectin is highly dependent on its structure which determines gelling characteristics, emulsion-stabilization activity and effect on viscosity. Thanks to the functions of it, pectin is not only used in food industry but also in pharmaceutical industry. Babbar et al. stated that pectin is made use of controlling the release of oligomers that acts as probiotic (2015).

Regarding the industrial application, one of the most common use of pectin is in jam industry for production of high sugar jams because the basic raw materials of jams are fruits and they already include pectin naturally. The additional pectin supplements the desired final product properties. The amount of additional pectin differs from one fruit to another depending on the naturally existing amount in fruit itself as it is shown in Table 1.1 (May, 1990). Jam production with high pectin containing fruits requires low additional pectin while higher amounts of additional pectin is used for jams of low pectin containing fruits.

Table 1. 1. Naturally existing pectin amounts in different fruits used in jam industry (May, 1990)

Low	**Medium**	**High**
Apple	Apricot	Cherry
Blackcurrant	Blackberry	Peach
Plum		Raspberry
Redcurrant		Strawberry

In the case of jellies, they are made from fruit concentrates that are depectinized. To improve rheological properties, required amount of pectin is completely additional pectin. However, addition of too much pectin makes the gel over strong which results in undesirable textural properties. The regulation of gel strength and texture are provided by pH adjustment. The difficulties of pH adjustment and gel formation are overcome by using low methoxyl pectin which is capable of forming strong gels even at high pH values. May (1990) indicates that high methoxyl pectin is said to be useful just for standard jams which have soluble solid content over 60 %. For production of reduced sugar jams which has 30 % or lower soluble solid content, low methoxyl pectins are preferred to reach excellent gel structure. That is to say, deciding the type of additional pectin is very critical for production of mentioned products because when the soluble solid content decreases, the appropriate pectin for production becomes more calcium sensitive in other words having lower methoxylation degree (May, 1990). The amount of fruit content in product is still critical in such a way that for very low soluble solid content, it is necessary to add calcium salts to obtain the desired gel structure.

Jam production industry produce also filling and topping products for products of bakery industry, but it is difficult to make a sweeping statement about the properties of pectin used for these purposes. The type of pectin is decided according to formulation of product and its special requirements according to product itself such as biscuit jams or jam tarts.

Another usage area of pectin in industry is seen in glazes used for pastry production and in flan production. The blends produced by pectin manufacturers are available for these products. Mixture, including low methoxyl pectin and sequestrants containing calcium, is formulated meticulously and can be used by diluting the mixture to obtain the glazes in clear and shiny form (May, 1990).

In recent years, after dairy industry includes the fruits in their production, the ingredients added to these products gain importance. Fruit bases are being added to dairy products like yogurts and the texture modification is provided by thickening agents. These agents should be capable of providing appropriate texture for filling the product in packages and protect the distribution of fruits inside the product even after filling process. Modified starch is an example of these thickening agents but the problem of it is blocking the fruit flavor. So, pectin is an effective option used for these products without a change in flavor. Depending on the sugar content of product, low or high methoxyl pectins are preferred again in this case. The ability of pectin stabilizing the protein structure of dairy products makes pectin advantageous for fruit juice and dairy product blends and soy based beverages. In such a case, aggregation of casein and precipitation of whey proteins are prevented, and stable final product structure is obtained. Moreover, thanks to its emulsifying ability, pectin finds an application in acidified milk drinks, fruit juices having high protein content and fortified foods high in antioxidant (Wicker et al., 2014). By acting as a fat replacer, it is used in production of ice creams, emulsified meat products and also spreads (Maran et al, 2013).

Regarding the production of soft drinks with low calorie values, thin texture and undesired mouth feel are general problems resulting from the lack of sugar. Textures of these products are modified with addition of even low amount of pectin and viscosity is increased to get desired mouth feel as close as possible to conventional soft drinks. Lower molecular weight pectins with respect to commercially available ones are better for foods having low viscosity especially beverages (Muhammad et al., 2014).

Except the wide usage area of pectin in food industry, it is also utilized in several applications in pharmaceutical industry especially for syrup production. The pectin standards used for these purposes are much more strict. Controlled viscosity, particle size and purest form of pectin are some of these requirements. May (1990) indicates that pharmaceutical industry accounts pectin not only as jam setting stuff but also as safe material having beneficial effects on human body. These effects can be summarized as cholesterol regulation in bloodstream, reduction in risk of heart diseases (Bagherian et al., 2011), inhibition of lipase activity (Kumar, 2010), inhibition of metastasis of cancer cells (Jackson et al., 2007). Munarin et al. (2012) state the effective usage of pectin for drug and gene delivery and tissue engineering.

1.4. Sugar Beet Pulp as Pectin Source

1.4.1. Sugar Beet Pulp Pectin

Sugar beet (*Beta vulgaris*) pulp is one of the most important food production wastes obtained from sugar production process. It includes 75 % (w/w) carbohydrates in dry matter, approximately. These carbohydrates are mainly glucose, arabinose and galacturonic acid. After the extraction of sugar from SBP, the dry matter content becomes 18-23 % (w/w) (Kühnel et al., 2011). Pectin forms the 10 - 30 % of this dry weight of sugar beet (Michel et al., 1985) but pectin percent is just 0.1 - 0.3 % of dry weight in SBP (Thakur et.al., 1997). SBP is an available source but it has low pectin content. So, it is generally not preferred as a source of pectin because of sugar beet pulp pectin having poor gelling properties. SBP, the waste stream, is utilized as animal feed in feed formulations with very low commercial values and environmental problems as a result (Huang et al., 2017). So, the utilization of this waste stream for production of valuable compounds is a hot topic in recent years (Chen et al., 2015).

The pectin structure obtained from SBP contains ferulic acids bounded to side chains, differently from citrus pectins (Rombout & Thibault, 1983). Moreover, pectin extracts obtained from SBP contains protein in the range of 2-10 % depending on the extraction conditions (Kirby et al., 2006). Addition to ferulic acid and protein, high acetylation degree is found in SBPP and this special composition makes it have superior emulsifying effect. SBPP gains hydrophilic property thanks to the carbohydrate structure and stabilize the emulsions by causing an increase in viscosity and steric effects (Nakauma et al., 2008). The protein fraction contributes to emulsifying effect of pectin by activating water-oil interphase (Akhtar et al., 2002). These properties make SBPP more advantageous hydrocolloid than pectins from other food sources.

1.4.2. Factors Affecting Pectin Content of Sugar Beet

Pectin is a biopolymer that majorly acts in water translocation in plant tissue. Amount of pectin present in plant is highly dependent on factors such as growing conditions of plant, harvesting time, storage duration and imposed upon treatment also have great effect on pectin that will be extracted from the plant source. So, a decrease in pectin quantity is seen as a result of ripening and softening due to enzymatic hydrolysis of pectin (Hook & Roboz, n.d). Unlike other food sources, sugar beet does not show much significance in pectin amount when it is harvested as mature beet or ripened beet which means seasonal variations stays less effective in terms of pectin content at harvest. However, the storage duration has the most significant effect comparing with other factors. Apart from this, considering the pectin extraction from waste streams of food industry, the processes discharge the waste that is used as a source of pectin also create considerable changes in pectin amount.

Immature fruit include pectin is named as protopectin and it is in the form of water insoluble hetero-polysaccharide (Inari et al., 2002). During maturation of fruit, decomposition of protopectin structure occurs and pectic enzymes hydrolyze the protopectin to water soluble form. Due to pectin being one of the major components

of plant cell wall in middle lamella, decomposition of pectin causes softening of fruit. As it is understood, the softening level during storage directly affects the quantity of pectin and refers to physiochemical changes in polysaccharides itself. Regarding that, the harvest time and storage duration characterize the extraction yield and properties of pectin being extracted.

1.5. The Way Forward to Characterization of Sugar Beet Pulp Pectin (SBPP)

1.5.1. Solvent-Based Pectin Extraction from Sugar Beet Pulp

Pectin extraction is the prior step for characterization of SBPP by removing the impurities such as destructed SBP cells, sugars in crystal structure and brown color pigments. Starting from the definition, solvent extraction is a method used for separation of compounds based on their solubility in special solvent which are differ from each other depending on the type of compound that is wanted to be recovered.

The most common solvent used for pectin extraction is water. Acidified medium with different mineral acids is employed by the support of elevated temperatures and continuous stirring conditions (Naqash et al., 2017). During solvent extraction, the hydrolysis of protopectin in plant cells and transformation of it to pectin turn the compound from water-insoluble form to water-soluble form. Actually, there are different supporting chemical agents used for extraction of pectin such as calcium ion chelators, bases, acids. However, acids show the strongest effect because they enable extraction of protopectin which is firmly bound to cell matrix and higher extraction yields are obtained comparing with other chemical agents (Sandarani, 2017).

1.5.2. Effect of Acid on Extraction

Several studies have indicated the effects of different acids on yield, functional properties and physiochemical properties of extracted pectin. In that case, the dominating characteristic of acid is strength of it, but type and concentration of acid also create variations. Malic acid, citric acid, hydrochloric acid, lactic acid, acetic acid, phosphoric acid and sulfuric acid are most commonly used acids for pectin extraction (Ma et al., 2013; Abbaszadeh, 2008; Michel et al., 1985). In all acid types, hydrochloric acid generally facilitates the highest extraction yield (Banu et al., 2012; Israel-Castillo et al., 2015). However, different plant sources may require different acids to reach maximum extraction yield.

Considering the hydrochloric acid, availability of hydrogen ions in high concentration triggers the protopectin hydrolysis and improves stabilization of pectin due to the ability of pectin for precipitating with cations like Ca^{+2}. However, acid including hot extraction media cause low esterification degree because of pectin structure being sensitive to strong acids (Chan & Choo, 2013). Nitric acid is another common acid that is used for acidification of extraction media for pectin extraction. While yield increases with decreasing the pH of media, structural properties of pectin vary as it is in the case of hydrochloric acid. It has found that the optimum pH in 1.2 for cinnamon pectin extraction (Besson et al., 2013). Sulfuric acid has given highest pectin yield for dragon fruit peel pectin (Tang et al., 2011). However, in the case of apple pectin extraction, significant results in extraction yield depending on different acid types could not be recorded (Yapo, 2011). The lowest extraction yields were recorded for pectin extraction from different plant sources for acidified extraction mediums by citric acid (Liew et al., 2014; Canteri-Schemin et al., 2005) while it gives the least pectin de-esterification degrees. So, citric acid is useful extracting agent to acidified medium in order to obtain pectin with good gelling properties.

1.5.3. High Hydrostatic Pressure (HHP) Application

1.5.3.1. General View

The first study for high hydrostatic pressure (HHP) has revealed in 1883 and it has been found that HHP may generate effects on organisms regarding the deep-sea ecosystems (Stal & Cretoiu, 2016). Hite (1899) studied about extending shelf life of milk by using pressure up to 650 MPa and this study was the first application of high pressure on food systems. In 1914, high pressure was employed for preservation of fruits and vegetables (Elamin et al., 2015). Until 1980s, the system has been developing and it has been found as an advantageous alternative to thermal food processes at last. Finally, in 1992, the first pressurized product found a place in market which was produced by a Japanese jam production company (Knorr, 1993). Today, HHP system finds various application areas in food industry for various purposes such as enzyme inactivation, reduction of microbial load, spoilage control, foaming of products, improvement of product properties for quality (High Pressure Processing of Foods, 2015). In summary, food industry employs HHP system for freezing and thawing, homogenization, pasteurization, sterilization and to assist thermal processes (Balasubramaniam et al., 2015).

HHP application in food industry has several advantages beside the point of having high installation cost. The system is regarded as novel non-thermal technology so the main advantage can be specified as overcoming or minimizing the negative effects of thermal processes. High temperatures to decrease and inactivate bacterial cells may cause undesirable flavor formation and loss in aroma and nutritional value of food materials. Application of high pressure instead of high temperatures or as a pretreatment helps to provide food safety without significant changes in physiochemical and quality characteristics of products (Huang et al., 2017). Lee et al. (2011) and Rastogi (2013) indicated that HHP is an influential technology to extend shelf life of food products that keeps heat labile components of food material like

vitamins without degradation and inhibit the off flavor formation while reducing the microbial load and inactivating microbial spores and enzymes. Except that, HHP system is accounted as clean technology and reduces or requires process time considerably (Parekh et al., 2017).

There are several studies in literature that isolate pectic polysaccharides from different plant sources by employing HHP and found HHP as advantageous method for extraction procedure. Naghshineh et al. (2013) carried out enzymatic pectin extraction by combining procedure with HHP and found that the combined process gives significantly (p<0.05) higher extraction results with higher degree of esterification than conventional-thermally extracted pectins at optimum pressure level i.e. 100 MPa. Moreover, they concluded that the HHP included procedure does not creates significant effect (p>0.05) on molecular weight and apparent viscosity of pectin which shows the HHP treatment having a high potential to be advantageous and sustainable process between all novel technologies. Guo et al. (2014) studied on novel methods for pectin extraction including HHP. They found that pectin extracted by HHP has the smallest particle size and provide 100% stable emulsions. Oliveira et al. (2016) stated the effect of combined process of HHP and conventional extraction of pectin from passion fruit where they found the yield was doubled by using HHP as pretreatment; so, they expressed this combined method as time saving, environment friendly and effective. Another study was conducted by Xie et al. (2018) about extraction of pectin from potato peel waste by HHP and high pressure homogenization. They suggested that HHP has improving effect on viscosity of extract and decreasing effect on degree of esterification; so, the technique was mentioned as efficient procedure for extracting pectin with modified structural properties.

1.5.3.2. Working Principle

The working principle of HHP system is to apply the same pressure to all points of sample at all directions at the same time for the same duration by the help of transmission of pressure by pressurization medium which is a liquid. This principle makes the process uniform. The pressurization liquid is mostly water regarding the applications in food industry; except that glycol or glycol-water and different oils may be employed in pilot scale applications. The pressurization liquid is selected considering the effect of pressure on its viscosity, compression ability under different temperatures and corrosion properties (Balasubramanian, 2003). The parameters of HHP system are pressure, temperature and time. A wide pressure and temperature ranges are applicable which are 100-1000 MPa and -20 – 100°C, respectively. The duration of pressure may be arranged from seconds to minutes above 20 min (Yaldagard et al., 2008).

The equipment consists of many parts which change in size and capacity depending on scale of process, product that will be pressurized and required process conditions. A standard system includes pressure vessel, pressure pump, end closures for covering pressure vessel, valves for controlling pressure, yoke for holding end closures stable under the pressure, intensifier for generation of determined pressure, process control equipment and product handling equipment for loading and removing product that will be pressurized (Balasubramaniam et al., 2015).

The operation of HHP application can be batch system, continuous system or semi-continuous system. Both solid and liquid products can be processed by batch system. After the load, pressurization liquid is isostatically pumped, pressure is applied until the desired value and after the determined duration of pressurization, compression on pressurization liquid is removed by the help of relief valve (Chawla et al., 2010). Regarding the continuous system, pressurization is only applicable for liquid products.

Pressure is applied in a tube with open end and through the end, product is decompressed. During decompression, heat generation occurs significantly because of friction and shear forces; for this reason, an uncontrolled thermal effect reveals (Cavender, 2011). To overcome this effect, semi-continuous systems are preferred. These systems work with continuous discharge. One vessel is pressurized and discharged; while discharging the first vessel, the second vessel is started to be pressurized and so on (Elamin et al., 2015).

1.5.3.3. Acting Mechanism and Effects of HHP

The effect of HHP treatment on pectin extraction is evaluated in two aspects: effect on yield and effect on pectin characteristics. The effect on yield is directly related with structural changes occurs when plant cell exposed to high pressure. High pressure creates an effective physical stress to break the cell wall even for pressure resistant cells and causes an irreversible cell damage (Alpas et al., 2003). Cell structure is fragile so that high pressure produces a destructive effect on cell membrane, denatures the protein structure of cell and causes cell deformation (Guo et al., 2012). When it is examined in detail, plant cell wall structure consists of dynamic networks of glycoproteins and hetero-polysaccharides that provide complexity and integrity to cell wall (Pogorelko et al., 2013). Moreover, cell membrane, which is fluid-like component, includes phospholipids involve proteins in their lipid matrix. Under high pressure, it loses motion of phospholipids. The more tightened packs and new gel-like structure of it makes the cell integrity damaged and more sensitive to physical stresses (Gonzalez & Barrett, 2010). As a result, the pectin, as hetero-polysaccharide in cell wall, becomes released easier than the conventional extraction method.

The effect of HHP on characteristics of already extracted pectin was studied by Peng et al. (2016) and it is concluded that high pressure affects the molecular weight and degree of esterification by leading depolymerization or chain breakage. Also,

stretched chain of pectins under high pressure results in an increase in viscosity. Guo et al. (2012) studied the ultra-high pressure effect on extraction of pectin from orange peel and concluded that there is no significant effect of high pressure on esterification degree and galacturonic acid content of pectin while stability of pectin is improved with pressure by an increase in activation energy because of an increase in inter and intra-interactions between pectin chains. Moreover, the mentioned study showed a significant increase in viscosity with respect to conventionally extracted pectin.

1.6. Characterization of SBPP

1.6.1. Extraction Yield

Estimation of pectin yield, which shows the efficiency of extraction, is calculated by taking the ratio of extracted pectin weight and SBP powder weight used for each extraction run, (% w/w) on dry basis. In order to determine the efficiency of extraction process, yield calculation is taken as most important variable for extraction processes. There are many researches in literature that is based on increasing efficiency by altering temperature-time combinations of process, inserting ultrasound assistance to process, inserting microwave assistance to process, using electromagnetic induction as assistant step, investigating dynamic pressure or high hydrostatic pressure effect on optimization of process (Yılmaz et al.,2016; Zouambia et al., 2014; Guo et al., 2014; Koh et al., 2014; Oliveira et al., 2016).

1.6.2. Degree of Esterification

Galacturonic acids are the main components in the pectin structure whose some of residues form ester bonds between methyl groups and free carboxyl groups. The amount of methyl esterified galacturonic acid residues denotes the degree of esterification of pectin sample. The esterification degree lower than 50 % indicates that pectin is low methoxyl pectin (LMP), while the esterification degree higher than

50 % implies the pectin is high methoxyl pectin (HMP). Degree of esterification has importance for determining functional properties of pectin sample and so commercial use of it.

To determine esterification degree, titrimetric methods have been used in most of research (Mesbahi et al., 2015; Yapo, 2009; Peng et al., 2016; Pinhero et al., 2008). The experimental method includes two titrations as before and after saponification of pectin. For both titration sodium hydroxide (NaOH) solution and phenolphthalein are used to maintain the pH of hydrolyzed and saponified polymer ester groups (Kiss et al., 2008). This chemical analysis includes multiple steps. The first step is initial titration which results in neutralization of free carboxyl groups of pectin molecules. The required titrant volume is recorded as initial titrant (IT). The second step is addition of specific amount of NaOH as alkaline solution and let the pectin to hydrolyze. Finally, an acid is added to solution in a calculated mol quantity according to NaOH in order to neutralize the NaOH used for hydrolysis purpose. Finally, the solution is titrated again with NaOH for released carboxyl groups in pectin solution and this titrant volume is recorded as final titrant (FT). The ratio of final titrant volume to totally used titrant volume in other words, esterified carboxyl groups over total carboxyl groups gives the degree of esterification.

1.6.3. Flow Behavior

Thickening and gelling properties of pectin create one of the widest usage areas of it in food industry as a texturizing agent so pectin is a valuable ingredient for food industry which helps to modify rheological properties of food systems. These properties indicate flow behavior, in other words viscosity and texture. Viscosity and texture of food product influence the sensorial properties so their modification with food additives gains importance. As concentration of pectin in a solution increases, each pectin molecule that tends to grab each other starts to contact and molecules

cannot move freely anymore. This transition from free movement to limited movement describes the mechanism behind the thickening ability of pectin in other saying the modification of viscosity. Concentration of pectin in solution, its molecular weight and degree of esterification create significance for flow behavior of pectin solutions. Chan et al. (2017) have found a linear relationship between shear stress and shear rate of solutions having pectin concentration up to 3% which means Newtonian flow behavior is observed up to this point. For higher concentrations, shear thinning behavior has been observed.

1.6.4. Galacturonic Acid Content

Galacturonic acid is a form of galactose which is composed with oxidation of D-Galactose. Its polymerized form, polygalacturonic acid, form the main chain namely backbone of pectic substance. Galacturonic acids are linked to each other to form α-(1-4)-D-Galacturonic acid units and neutral sugars bound to this backbone as side chains (Mohnen, 2008) which as a result indicates the pectin structure. The ratio of Gal-A to complete molecule gives the Gal-A content of pectin. Gal-A content and gelling ability of pectin molecule are related to each other so the percent of it gives idea about the function of pectin. Moreover, low galacturonic acid content may be interpreted as high degradation of pectin throughout the extraction process. In order to categorize pectin molecule as functional food additive, FAO requires that it should contain at least 60-65% Gal-A (Food and Agricultural Organization). So, the Gal-A content of pectins extracted from different sources with many different extraction processes becomes an important variable to decide on functional properties of polymer.

Gal-A content can be determined by different experimental methods according to literature. Yılmaz et al. (2016) determined the Gal-A content by HPLC after enzymatic modification of sugar beet pulp pectin. Although HPLC is an efficient quantification

method in the case of presence of more than one sugar type in sample, it is disadvantageous for large samples of only one sugar type (Taylor, 1993).

James et al. (1952) used a photometric determination method which is named as Carbazole Method. Carbazole reagent in sulphric acid is used to develop chemical reaction specific to galacturonic acid. Strong acid destroys the polymer and reagent react with galacturonic acid which is then read by spectrophotometer.

Naghshineh et al. (2013) detected the Gal-A % spectrophotometrically by m-hydroxydimethyl method. This method has the same principal with Carbazole method, but the reagent used in there is meta-hydroxy-diphenyl. This method is more sensitive than Carbazole method, so the researches of recent years includes this method in order to determine Gal-A content. In this study, galacturonic acid content was obtained by m-hydroxydiphenyl method as it was explained by Blumenkartz & Asboe-Hansen (1973) with a slight modification.

1.6.5. Fourier Transform Infrared Spectroscopy (FTIR) Analysis

The molecules and different chemical bonds such as -OH, -CH, proteins, non-esterified and esterified carboxyl groups were seen by analyzing FTIR Spectroscopy and commented on these spectral regions in order to recognize pectins obtained by different experimental parameters. Although the region that can be named as fingerprint of carbohydrate molecules is between 950-1200 cm^{-1}, the structural changes occur in pectin molecule are analyzed by observing different spectral regions between 600-3500 cm^{-1} (Cerna et al.,2003). The bands around 2000-2400 cm^{-1} indicates the moisture absorbed by pectin molecules. The board around 3000-3500 cm^{-1} refers to -OH bonds, 2750-3000 cm^{-1} shows the -CH bonds. Esterified and non-esterified carboxyl groups are linked to 1700-1750 cm^{-1} and 1600-1650 cm^{-1},

respectively. Moreover, the bands between 1500-1550 cm^{-1} informs of presence of proteins in pectin molecule structure (Huang et al., 2017).

The peaks at different spectral regions of pectin was used in characterization of pectin quality and its structural changes after variety of treatments in many researches (Manrique & Lajolo, 2002; Vasco-Correa et al., 2017; Zouambia et al., 2017; Grassino et al., 2016). Manrique and Lajolo indicate that the intensities of peaks that shows esterified carboxyl groups and non-esterified carboxyl groups can be used to analyze esterification degree of pectin molecule by proportioning (2002). Zouambia et al. shows that comparing the peaks at specific spectral regions of pectin molecules of extracted pectin and a commercial pectin can denote the effectiveness of extraction method (2017). Grassino et al. reveals the effect of ultrasound treatment on structural changes of pectin by analyzing the FTIR spectra of pectin samples extracted by conventional and ultrasound assisted extraction methods (2016). Peng et al. shows the effect of HHP on structure of pectin by measuring FT-IR spectra and changes in stretching vibrations of different bonds and molecules in pectin structure (2016).

1.7. Objectives

The scope of this study is to see the effect of high hydrostatic pressure on pectin extraction process and on quality characteristics of extracted pectin. Starting from this point, the main aim is to increase pectin extraction yield with respect to conventional - thermal pectin extraction process. HHP is thought to be a factor that could improve the extraction process by reducing extraction temperature and/or extraction time while still resulting in higher extraction yields with respect to conventional extraction. On the other hand, the structural and rheological properties of extracted pectins were also determined to see effect of HHP on function of pectin in order to understand the industrial value of extracted pectins in this study.

CHAPTER 2

MATERIALS AND METHODS

2.1. Materials

Sugar beet pulp, as the waste of sugar production process, was obtained from Kayseri Şeker (Kayseri, Turkey). The pulp was still wet, so it was dried to reduce water activity and make it possible to obtain pulp powder by using grinder. After the sugar beet pulps were dried in drying oven, they were grinded to obtain sugar beet pulp powder for further use. Hydrochloric acid (HCl), ethanol (C_2H_5OH), acetone (C_3H_6O), phenolphthalein ($C_{20}H_{14}O$), sodium hydroxide (NaOH), sulphric acid (H_2SO_4), sodium tetraborate ($Na_2[B_4O_5(OH)_4].8H_2O$), m-hydroxydiphenyl ($C_{12}H_{10}O$), and galacturonic acid ($C_6H_{10}O_7$) were bought from Sigma-Aldrich Chemical Co. (St. Louis, Missouri, USA). For comparison of analysis results of extracted pectins in this study, industrial sugar beet pectin was obtained from Kayseri Şeker (Kayseri, Turkey) and used as standard pectin.

2.2. Methods

2.2.1. Sugar Beet Pulp Pectin (SBPP) Extraction

2.2.1.1. Sugar Beet Pulp Powder Preparation

Sugar beet pulps were dried to preserve them during study duration. The drying was provided by using drying oven at 105°C for approximately 2 days, until the weight became constant. After drying, the pulps were grinded to obtain pulp powder at low particle size which will enable to obtain higher pectin yield in pectin extraction (Ma et al., 2013).

2.2.1.2. Conventional Extraction

Conventional extraction was performed by using 3 extraction temperatures which are 70°C, 80°C and 90°C. The extraction time and pH condition for extraction medium was based on literature data. According to Yılmaz et al. (2016), higher extraction yields was observed at 1.2 pH and 5 hours extraction parameters regarding many times, temperature, pH combinations as extraction parameters. Moreover, Yılmaz et al. (2016) indicates that the pulp powder-water mixing ratio and ethanol volume added to extraction medium to precipitate extracted pectins are also having great importance on pectin extraction yield. Therefore, mixing ratio and ethanol volume were decided as 1:10 (w/w) and 1:3 (v/v), respectively as a result of literature review (Zaid et al, 2016; Yılmaz et al, 2016). Conventional extraction was conducted as it can be seen in Figure 2.1, flow chart of conventional extraction for 90°C – 5 h. Extractions with other temperature-time combinations were conducted by changing temperature and time parameters at the third step in Figure 2.1.

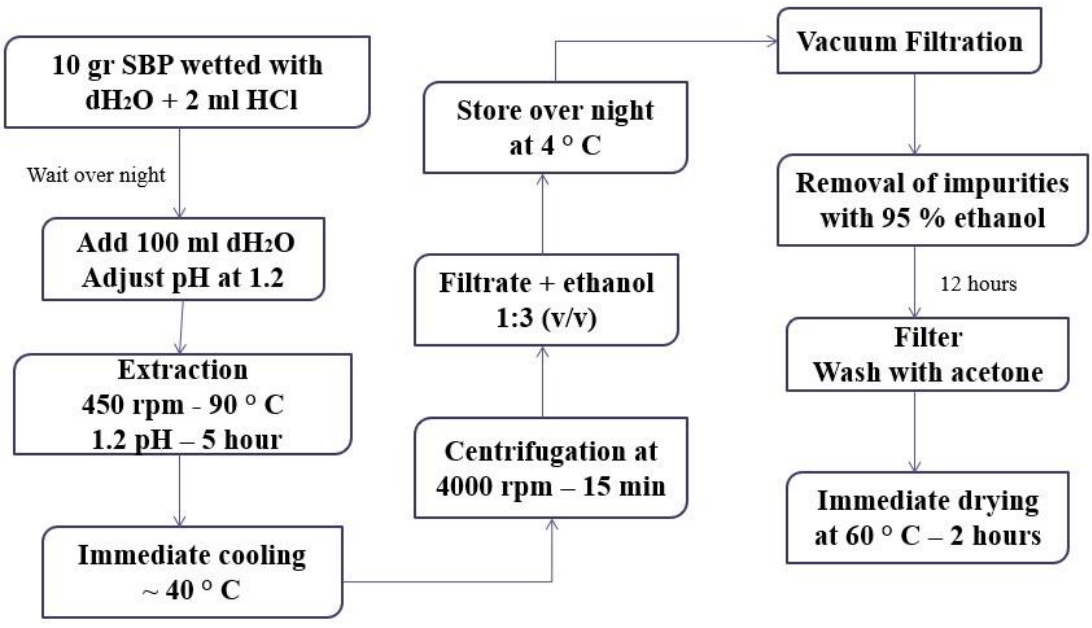

Figure 2. 1. Flow chart of conventional extraction for 90°C – 5 h parameters

Conventional extraction was conducted as it can be seen from Figure 2.1, flow chart of conventional extraction. In the procedure, 10 gr sugar beet pulp powder was wetted with distilled water and 2 ml of HCl was added. The mixture was waited over night before extraction in order to let acid destroy the cell wall and make pectin release from cell wall easier. Then, extraction was performed by using magnetic stirrer at 450 rpm for 5 hours and temperature was adjusted at 90°C. pH adjustment was provided by using HCl again and pH was kept constant at 1.2 during extraction. When 5 hours were over, samples were cooled to 40°C and centrifugation was performed at 4000 rpm for 15 minutes in order to precipitate the pulp powder while pectin was in a form of dissolved material in liquid part. After centrifugation, liquid part was mixed with ethanol at 1:3 (v/v) ratio and waited at 4°C - over night to provide for pectin precipitation. The cooled mixture over night was filtrated by using vacuum filtration method. Pectin and impurities collected of filter paper were recovered from filter paper and 20 ml of 95 % ethanol was added on them for removal of impurities. The mixture was kept at 60°C drying oven to evaporate ethanol and water for 12 hours. Dried samples were washed with acetone several times and finally dried again at 60°C drying oven until the weight is fixed, approximately 2 hours.

2.2.1.3. HHP Assisted Extraction

HHP treatment was conducted with 760.0118 type pressure equipment (SITEC-Sieber Engineering, Zurich, Switzerland). The vessel had 100 mL volume, 24 mm ID and 153 mm length. Heating–cooling system was built-in where it was maintained and controlled the temperature (Huber Circulation Thermostat, Offenburg, Germany). Temperature measurement was provided by K type inside vessel. The vessel was filled with distilled water as pressure transmitting medium. Extraction medium were poured into 25 mL sterile polyethylene cryotubes (LP Italiana SPA). The HHP equipment was given below in Figure 2. 2.

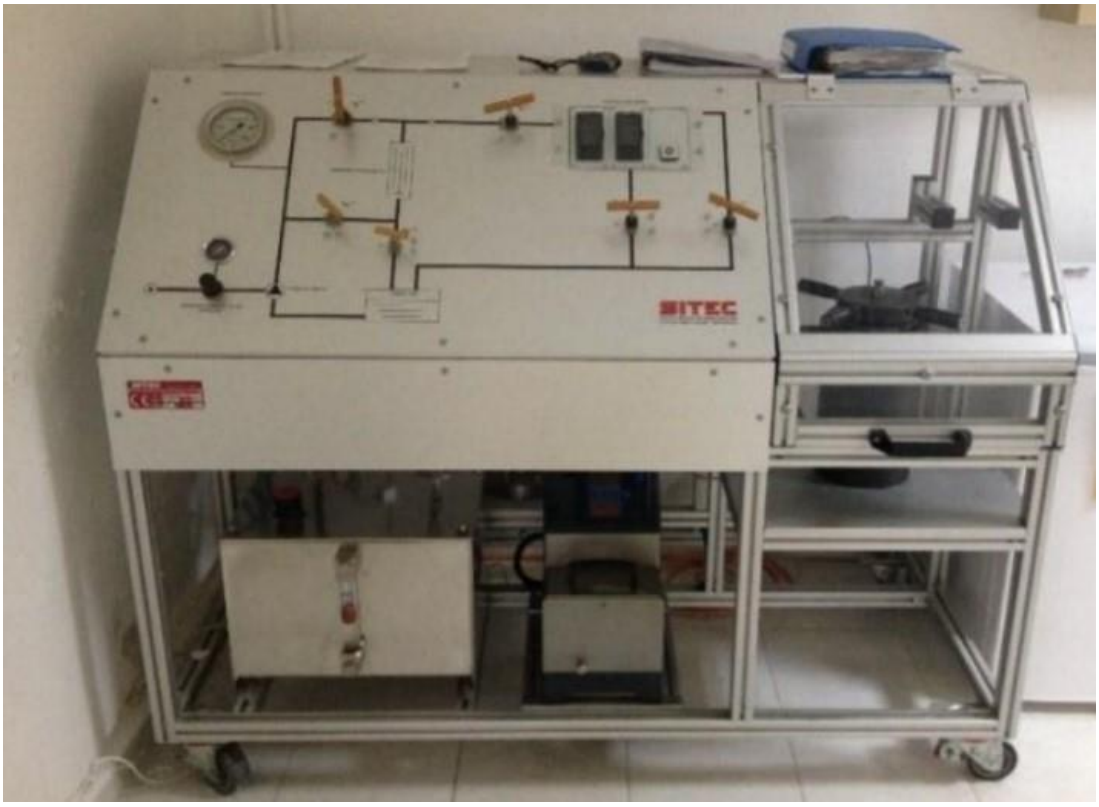

Figure 2. 2. HHP Equipment

For this method, conventional extraction was performed exactly the same just after HHP treatment. However, two different procedures were followed. For the first procedure, 2 ml of HCl was added to extraction medium and the HHP was applied to this medium to destroy cell wall. Three different pressures were applied as 250, 350, 450 MPa and pressure was applied at 40°C for 5 min. After pressure treatment was done, extraction was performed by using magnetic stirrer at 450 rpm for three different extraction times (3, 4 and 5 hours), two different temperatures (80°C and 90°C). After finishing the extraction, samples were centrifuged at 4000 rpm for 15 min and liquid part was taken. The liquid part was mixed with 1:3 (v/v) ratio ethanol and waited at 4°C - overnight. Then, vacuum filtration was proceeded, and the part remained on filter paper was collected to add 20 ml ethanol on it for impurity removal. To evaporate

water and ethanol, 60°C drying oven was used, and dried samples was washed several times with acetone to obtain pure pectin.

For the second procedure, extraction medium was prepared by mixing 10 gr of pulp powder and 100 ml distilled water, only. Acid addition was not performed in this step. The pulp powder-distilled water mixture was treated with three pressures (250, 350, 450 MPa) at 40°C for 5 min. After the pressure treatment were done, acid addition was implemented, and pH was adjusted at 1.2. Then extraction was performed by remaining steps the same as the first procedure.

2.2.2. Characterization of Sugar Beet Pulp Pectin (SBPP)

2.2.2.1. Determination of Extraction Yield

Extraction yield indicates the amount of pectin obtained at the end of extraction and following purification steps and it is an important indicator of extraction efficiency. The extraction yield is proportion of initial amount of sugar beet pulp powder that will be used for extraction and final amount of pectin that has been obtained. So, calculation was done by the ratio of extracted pectin weight and SBP powder weight used for extraction on dry basis (% w/w) as it can be seen in Eq. 1 below:

$$\text{Extraction Yield (\%)} = \frac{\text{dry weight of extracted pectin}}{\text{dry weight of sugar beet pulp powder}} \text{ x } 100 \qquad \text{(Eq. 1)}$$

2.2.2.2. Determination of Degree of Esterification

To determine esterification degree, the same titrimetric methods have been slightly modified and used in most of research (Mesbahi et al., 2015; Yapo, 2009; Peng et al., 2016; Pinhero et al., 2008). This titrimetric method that includes two titrations as

before and after saponification of pectin was used in this study, too. The esterification degree of pectin obtained from each extraction run was determined as described by Peng et al. (2016). 0.2 g of pectin samples was wetted by ethanol and 20 ml distilled water. Complete dissolution was provided by the help of ultra turrax. 3 drops of phenolphthalein was added to solution and mixture was titrated with 0.5 M NaOH until permanent pink color is developed. Thus, titration before saponification was completed and titrant volume, the NaOH volume used for titration, was recorded as V_1. Then, 10 ml of 0.5 M NaOH was added to solution and it was continuously stirred for 30 min duration. At the end of 30 minutes, 10 ml of 0.5 M HCl was added to solution and stirred until the pink color disappears. The second titration was performed, and solution is titrated by 0.5 M NaOH again. At this step, titration after saponification was completed and titrant volume was recorded as V_2. Degree of esterification was calculated by substituting the titrant volumes V_1 and V_2 in the following equation:

$$DE\ \% = 100 \times [V_2 / (V_1+V_2)] \qquad \text{(Eq. 2)}$$

It was not expected to see significant change in degree of esterification of extracted pectin of each extraction run in this study because of studying in acidic conditions. According to Michel et al. (2001), degree of esterification shows significant changes in alkali extraction conditions.

2.2.2.3. Determination of Flow Behavior

The flow behavior of pectin solutions was determined by measuring their viscosity. Pectin samples were dissolved in distilled water as 2g/L and all measurements were done at 25°C. For measurement, Kinexus dynamic rheometer was used (Malvern, Worcestershire, UK) with its concentric cylinder geometry. Shear rate values required

to set up the shear rate ramp were selected as 0.1 s^{-1} start shear rate and 100 s^{-1} end shear rate. Viscosity vs. shear stress data were analyzed and viscosity of each solution is recorded that have Newtonian behavior.

2.2.2.4. Determination of Galacturonic Acid Content

The galacturonic acid content was determined by using the experimental method that takes its name from the reagent used for determination, m-hydroxydiphenyl method. This method was described in detail by Blumenkrantz and Asboe-Hansen and followed in most of uronic acid determination studies in literature (1973). The sample preparation and spectrophotometric measurement procedure was followed as its in study of Zouambia et al. (2017) with slight modification. 10 mg pectin was dissolved in 10 ml of distilled water and 800 µl sample of this solution was mixed with 4.8 ml of 0.125 M sodium tetraborate in H_2SO_4. The mixture was waited in boiling bath for 5 min and cooled in ice bath. Then, 80 µl of 0.15 % m-hydroxydiphenyl in 0.5 % NaOH was added to solution and stirred. Pink color started to develop, and 5 min duration was waited to obtain permanent color. As a final step, absorbance values of samples were measured in UV-Spectrophotometer at 520 nm. In order to obtain interpretable data for each pectin sample, the absorbance values should be converted to galacturonic acid concentration values. For this purpose, calculation requires a graph called standard curve. Standard curve was formed by using standard galacturonic acid solutions in different concentrations as 25, 50, 75, 100, 150, 200 µg/ml. The absorbance values of this solutions were measured in UV-Spectrophotometer at 520 nm and a standard curve in Appendices A was obtained for Gal-A determination. The equation obtained from this graph was used to calculate Gal-A concentrations of each solution from their absorbance values.

2.2.2.5. Fourier Transform Infrared Spectroscopy (FTIR) Analysis

The molecules and different chemical bonds such as -OH, - CH, proteins, non-esterified and esterified carboxyl groups were seen by analyzing FTIR Spectroscopy. The bands around 2000-2400 cm^{-1} indicates the moisture absorbed by pectin molecules. The board around 3000-3500 cm^{-1} refers to -OH bonds, 2750-3000 cm^{-1} shows the -CH bonds. Esterified and non-esterified carboxyl groups are linked to 1700-1750 cm^{-1} and 1600-1650 cm^{-1}, respectively. These spectral regions were commented in order to recognize pectin samples obtained by different experimental parameters. IR Affinity 1 Spectrometer with ATR attachment were used for measurement (Shimadzu Corporation, Kyoto, Japan). Replicates of each sample were read twice in the band of 500-4000 cm^{-1} with 4 cm^{-1} resolution and 32 scans as measurement parameters. The FTIR device was given below in Figure 2.3.

Figure 2. 3. Fourier Transform Infrared Spectroscopy (FTIR) device

2.2.2.6. Water Holding Capacity with Nuclear Magnetic Resonance (NMR) Relaxometry

Water holding capacity of pectin solutions were analyzed by measuring spin-spin relaxation time, T_2. For this measurement, Spin Track NMR Relaxometry instrument was used. The magnetic field of device is 0.5 Tesla and frequency is 20.34 MHz. CPMG (Carr-Purcell-Meiboom-Gill) pulse sequence was used to record T_2 relaxation with selected parameters of selected as 2000 ms echo time, 3000 echo number, 10.000.000 time of observation, 3000 s repetition time and 16 number of scans. Moreover, relaxation time was selected as 11000. The NMR Relaxometry device was given below in Figure 2. 4.

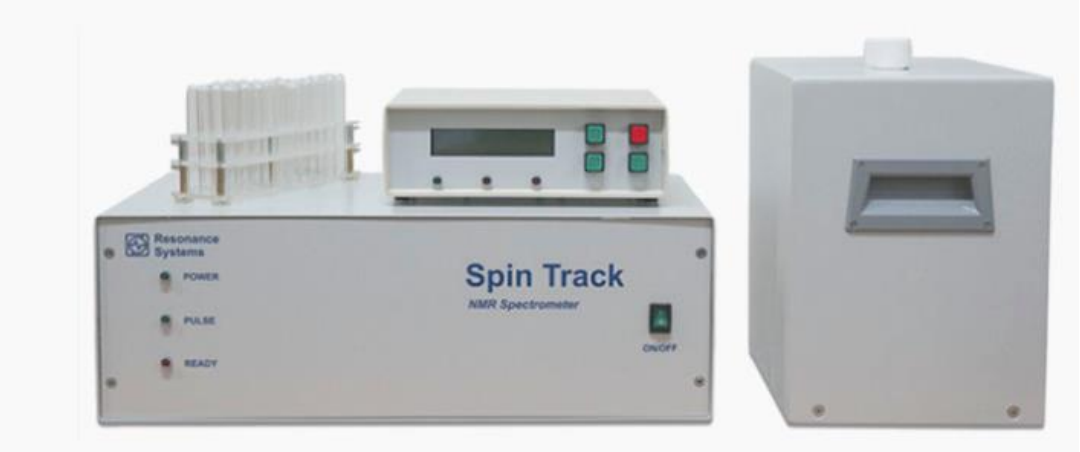

Figure 2. 4. Nuclear Magnetic Resonance (NMR) Relaxometry device

2.2.3. Statistical Analysis

Statistical analysis was performed for all determinations of the experiment by use of MINITAB (Version 16.1.0.0, Minitab Inc., Coventry, UK).

In order to see the significance of difference in dependent variables of experiments, Analysis of Variance (ANOVA) was carried out. The change in pressure of HHP treatment, extraction temperature and extraction duration create difference in pectin extraction yield, DE value of extracted pectin, viscosity of solutions prepared by extracted pectin and Gal-A content of extracted pectin were examined by practicing ANOVA. Through the multiple comparison tests, Tukey's test was performed with 95% confidence level, $p = 0.05$. Experimental results were obtained as replicate and sample number for analysis was thirty-six ($n = 36$). The conditions relevant to number of level and factor were given in Table 2.1.

Table 2. 1. Representation of factors and levels of the study

Factors	**Levels / Conditions**
Pectin Extraction Methods	Conventional Extraction HHP Assisted Extraction
Acid Addition Step	Pressurization with Acid Pressurization without Acid
Pressure Levels	250 MPa, 350 MPa, 450 MPa
Extraction Temperatures	80°C, 90°C
Extraction Times	3 h, 4 h, 5 h
Extraction pH	1.2

CHAPTER 3

RESULTS AND DISCUSSION

All characterization parameters including extraction yield, DE value and Gal-A content of extracted pectin, flow behavior of solutions prepared by extracted pectin, peaks in FTIR Spectroscopy and water binding capacity obtained by using NMR Relaxometry were obtained as differing values according to different pressure values of HHP treatment (250, 350, 450 MPa), different extraction temperatures (80°C, 90°C), different extraction times (3, 4, 5 h) and acid addition before or after HHP application. The samples treated with HHP after addition of acid are named as *'Samples pressurized with acid'* and the samples treated with HHP before acid addition are named as *'Samples pressurized without acid'* through remaining parts of the thesis.

3.1. Extraction Yield

3.1.1. Pectin Yield of Conventional Extraction Method

Control experiment was performed as conventional extraction in order to see the pectin extraction yield from sugar beet pulp powder differing as regards two different temperatures (80°C, 90°C) and three different extraction durations (3, 4, 5 h). Maximum extraction yield was obtained at highest durations for both of 80°C and 90°C extractions as 5.68 ± 0.02 % and 6.43 ± 0.07 %, respectively where the maximum extraction yield was observed at the extraction conditions of 90°C for 5 h. The obtained conventional extraction yields were consistent with the study of Yılmaz et al. (2016). The yield results which show the effect of extraction temperature and time on pectin extraction yield are given in Figure 3.1.

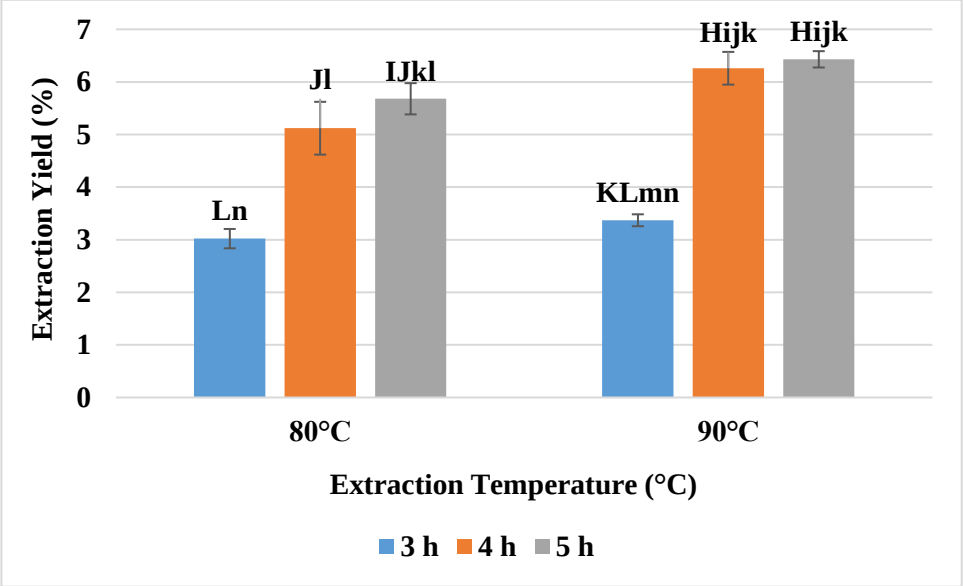

Figure 3. 1. Experimental results of pectin extraction yield by following conventional extraction procedure

Different letters denote significant difference (p<0.05). Uppercase letters represent significant difference of samples pressurized with acid while lowercase letters represent statistical analysis of samples pressurized without acid.

3.1.2. Pectin Yield of HHP Assisted Extraction Method

Pectin yields were calculated for each of different pressure-temperature-time combination. Besides, each combination was repeated regarding to acid addition step as before pressurization and after pressurization. Maximum extraction yield was obtained at highest pressure, extraction temperature and duration for both pressurized with acid and without acid samples as 12.23 ± 0.13 % and 12.09 ± 0.11 %, respectively where the maximum extraction yield was observed for pectin samples obtained with pressurizing at 450 MPa with acid and extracting at 90°C for 5 h. The related results for samples pressurized with acid and samples pressurized without acid are given in Figure 3.2 and Figure 3.3, respectively.

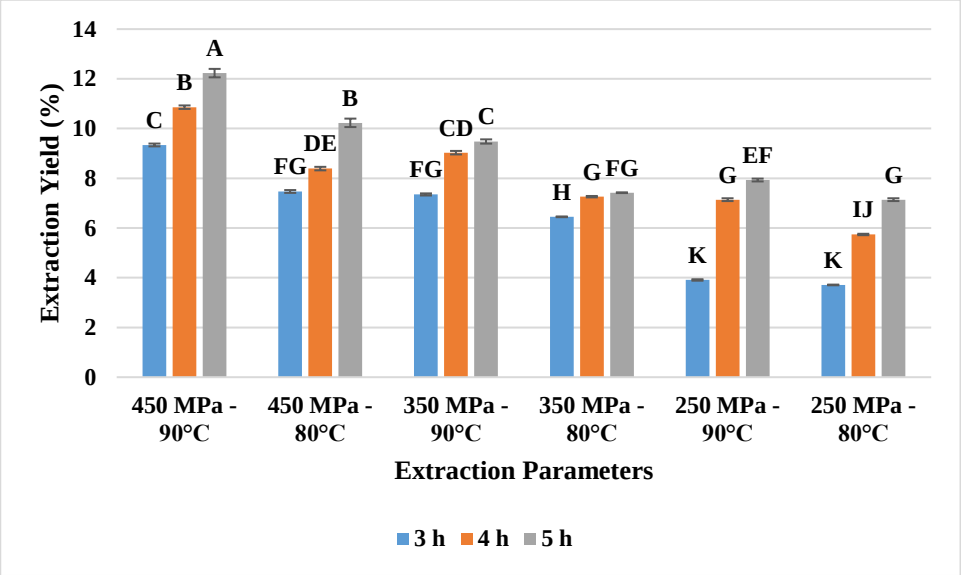

Figure 3. 2. Experimental results of pectin extraction yield by following HHP assisted extraction procedure for samples pressurized with acid

Different letters denote significant difference (p<0.05).

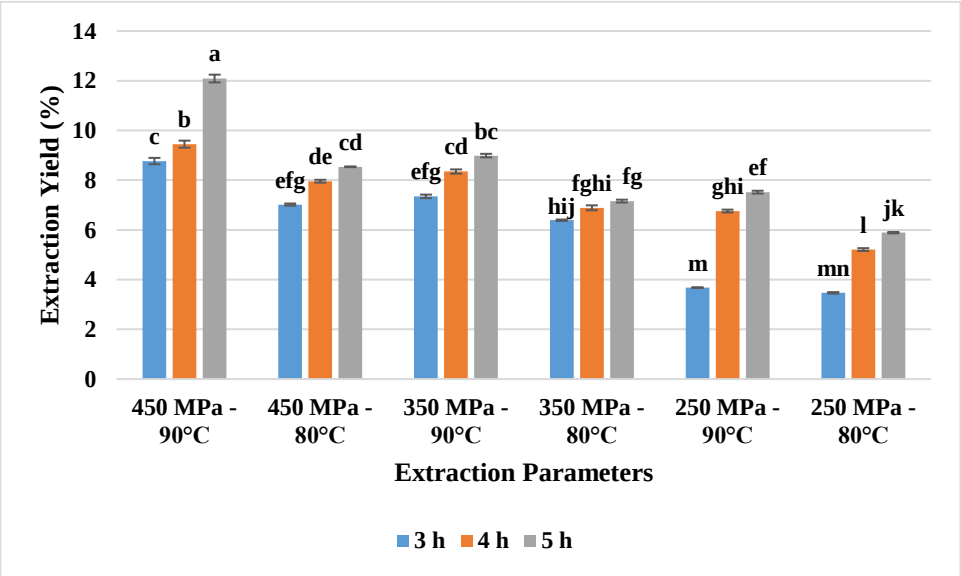

Figure 3. 3. Experimental results of pectin extraction yield by following HHP assisted extraction procedure for samples pressurized without acid

Different letters denote significant difference (p<0.05).

Moreover, the results of all statistical analyses for extractions at different pressure, temperature and time values and acid addition step can be seen from ANOVA outputs found in Appendix C.

Regarding conventional extraction, it was found that 80°C – 3 h and 90°C – 3 h extraction yield was significantly lower than the yield results of 350 MPa and 450 MPa pressure levels ($p \leq 0.05$). However, at 250 MPa – 3 h results for both experimental group, yields are close to conventional extraction yields at 80°C – 3 h and 90°C – 3 h which means applying 250 MPa pressure is not enough to increase extraction yield at short extraction duration such as 3 hours. By looking at 90°C – 5 h extraction yields also, it was observed that pressure levels higher than 250 MPa and extraction durations at least 4 h or more are required to reach yields close or higher than 90°C – 5 h yields but it should be considered that including high hydrostatic pressure to process is replace high temperature (90°C) and/or long process times (4 and 5 h) to reach close yield to higher conventional extraction yield.

According to 1-way ANOVA results, all of the tree independent variables which are pressure, temperature, time showed significant difference in themselves for both samples pressurized with and without acid ($p<0.05$). However, samples pressurized with acid showed bigger difference and time became more critical parameter with respect to samples pressurized without acid. The reason for that acid and pressure show synergistic effect together on triggering the cell destruction. Considering overall means for these three variables, the results of two experimental groups which are samples pressurized with acid and samples pressurized without acid, were close to each other in the case of temperature.

According to 2-way ANOVA results, for different combinations of pressure-temperature, 450 MPa - 90°C gave the highest extraction yield significantly (p<0.05). The other pressure-temperature combinations were also significantly different except for 450 MPa - 80°C and 350 MPa - 90°C data for samples pressurized with acid (p>0.05). These two combinations did not show significant change, so it can be said that increasing the pressure provide high extraction yield at lower temperature. However, for samples pressurized without acid, all P-T data are significantly different (p<0.05) which means each of every parameter become more critical. For both experimental group, highest yield was at 450 MPa - 90°C combinations and lowest yield was at 250 MPa - 80°C combinations. Samples pressurized with acid have higher yield for comparison of almost all P-T-t combinations because of mentioned synergistic effect. Alpas et al. (2003) indicated that pressure effect on cells depends on the change of volume at ambient pressure and HHP due to the lower volumes are favored at high pressure levels. They also showed that high pressure levels cause conformational changes in cell structure due to pressure shift in dissociation-association equilibrium toward dissociation. While this effect is combined with existence of acid in medium, elevated destructions of cell structure result which was characterized as synergistic effect of pressure and acid. After the pressurization, SBP cells become more sensitive to heat and it makes pectin release much easier. For different pressure-time combinations, all combinations were significantly different ($p<0.05$) with the exception of 350 MPa – 5 h, 350 MPa – 4 h and 450 MPa – 3 h for both experimental groups ($p>0.05$). The highest yield was at 450 MPa – 5 h. Increasing the high hydrostatic pressure level that was used as assisted method to extraction, indicates higher yields even at lowest extraction times. In other word, pressure replaces the time and play a bigger role on increasing efficiency of process than time. The same comment is valid for samples pressurized without acid and the similarity (p>0.05) of yield results at 350 MPa – 3 h and 250 MPa – 5 h is proof of this comment. Comparing the different temperature-time combinations, the yield results were significantly different from each other (p<0.05). Highest yield was at 90°C – 5 h combinations and the lowest yield was at 80°C – 3 h combinations. The

yield results of 90°C stayed higher than 80°C – 5 h for all extraction times so it can be said that temperature is more critical factor than time for both experimental groups.

According to 3-way ANOVA results, the highest yields for both experimental groups were at 450 MPa - 90°C – 5 h combinations between all combinations and 450 MPa - 90°C – 3 h results were not significantly different from 350 MPa - 90°C – 5 h (p>0.05). These data prominently show that extraction time can be reduced dramatically by high hydrostatic pressure application. This result was also consistent with the study of Oliveira et al. (2016). Similarly, 450 MPa - 90°C – 3 h and 350 MPa - 90°C – 4 h results were also not show significant difference (p>0.05).

In the case of samples pressurized with acid, there is no significant difference (p>0.05) between 350 MPa - 80°C – 4 h, 350 MPa - 80°C – 5 h, 350 MPa - 90°C – 3 h and 450 MPa - 80°C – 3h combinations. These results proved that pressure at the studied levels provides higher extraction yields even at the lowest T-t combination, i.e. 80°C – 3 h. Moreover, 450 MPa pressure tolerates decreases in both temperature and time while producing higher efficiencies. Another quadruplet combination that their results showed no significant difference (p>0.05) were 350 MPa - 90°C – 3 h, 350 MPa - 80°C – 4 h, 250 MPa - 90°C – 4 h and 250 MPa - 80°C – 5 h combinations. Overall, when the applied pressure is increased by increments of 100 MPa from 250 to 350 MPa and then to 450 MPa, the same yield of the process is reached at 1 h shorter time.

For samples pressurized without acid, the data did not show significant difference (p>0.05) and pressure replaced both temperature and time were 450 MPa - 80°C – 3 h, 350 MPa - 80°C – 4 h and 250 MPa - 90°C – 4 h combinations. However, yields of samples pressurized with acid were higher at these parameters. This is mainly due to,

both pectin molecules released during pressurization and released during heat treatment faced with acid and heat at the same time and degraded.

All in all, it was seen that pressure became as the most critical parameter in extraction process assisted with high hydrostatic pressure with respect to temperature and time. High pressure compensates the extraction yield difference in both decreasing temperature and time as a result it improves the extraction process. Action mechanism behind this situation is accelerated cell damage of SBP powder structure. Cell membrane of SBP powder is fluid like material with phospholipids and proteins in its matrix. When facing high pressures, this membrane loses its fluid like motion and behaves like a gel due to its packs becoming more tightened. This 'new form' of membrane structure impairs the cell's integrity and sensitivity of cell to any developing physical damage (Gonzalez & Barrett, 2010). As a result, pectin is released more easier than conventional extraction. In the case of samples pressurized with acid, both acid and pressure destroy the cell structure and quicken the pectin release even at *pressurization step* and help the heat treatment to be effective in a shorter time at *extraction step*. Thus, higher efficiencies are obtained because already extracted pectin molecules are not exposed to acid and temperature for prolonged extraction durations.

3.2. Degree of Esterification (DE)

Firstly, it should be remembered that pectin molecule is a methyl esterified polymer of D-Galacturonic acid. This molecule creates a structure that strengthens the cell wall. However, extrinsic factors cause breaking off methyl groups and replacing it with a -OH group. Thus, esterified carboxylic acid content of pectin decreases which is identified as a decrease in esterification degree, namely DE value. It is predicted that DE value will be low in this experimental design due to acidic extraction conditions and formation of fractions in polymer structure. Regarding samples pressurized with or without acid, it is seen that DE value of pectin is affected a bit from acidity of

environment during pressurization step because in this step, pectin is released even a little. This is because saponification process being catalyzed by hydroxyl ions. The pressurization at environment without acid, by deesterification of pectin in high pH value, ester bonds break down and replace with -OH bonds which are predominate in environment. As a result, DE value decreases. Considering that under high hydrostatic pressure, even a little part of pectin yield comes from pressurization process as assistant step, the results found by Michel and Autio (2002) matches up with results of this study. The acid added after pressurization is included in an environment that already have a fair amount of pectin that are deesterified even at pressurization step and continue to deesterify by fraction of side chains during heat treatment.

Regarding the DE values of pectin extracted with conventional extraction, it was higher at conventional extracts than samples pressurized without acid while it was close to 250 MPa pressurized samples for samples pressurized with acid (p>0.05). The values of conventional 80°C – 5 h and 90°C – 4 h results showed no significant difference (p>0.05) in between and this DE values were not significantly different than 5 h results of all high pressure levels. It means that effect of pressure reveals with prolonged heat exposure time to heat treatment and decrease of DE value is balanced as pressure increases with released pectin towards the end of extraction. The related results for conventional extraction, and two groups of HHP assisted extraction which are samples pressurized with acid and samples pressurized without acid are given in Figure 3.4, Figure 3.5 and, Figure 3.6 respectively.

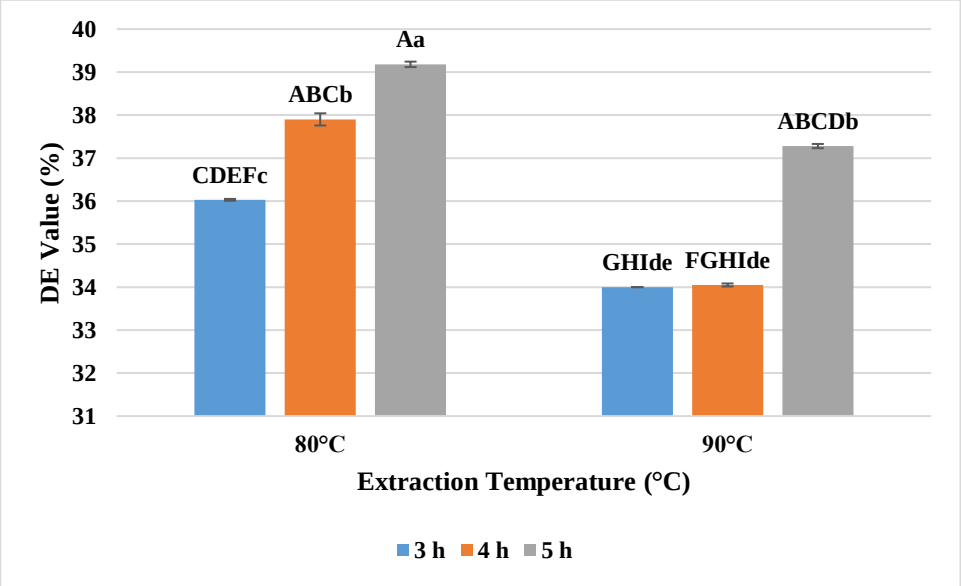

Figure 3. 4. Experimental results of DE Value of pectins extracted by conventional extraction

Different letters denote significant difference (p<0.05). Uppercase letters represent significant difference of samples pressurized with acid while lowercase letters represent statistical analysis of samples pressurized without acid.

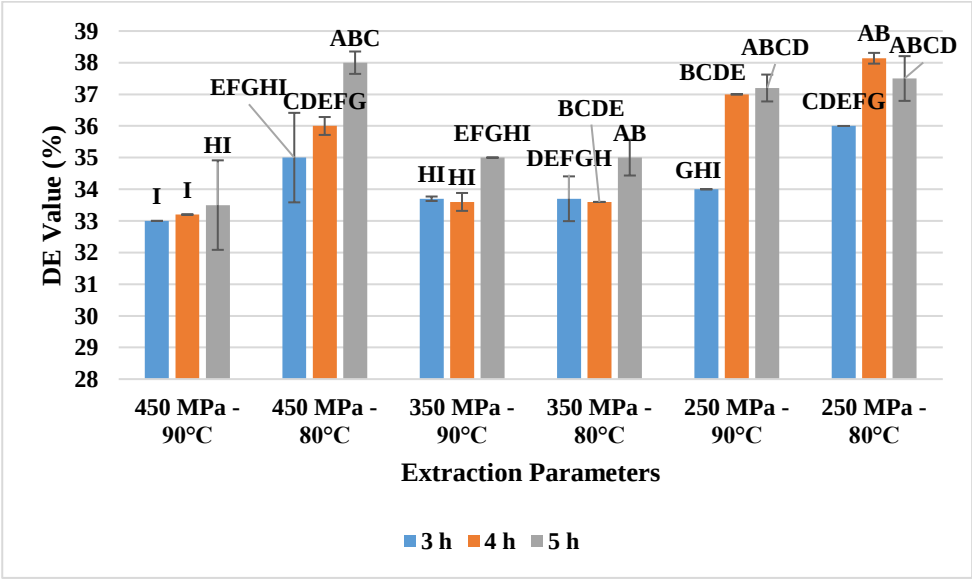

Figure 3. 5. Experimental results of DE Value of pectins extracted by HHP assisted extraction for samples pressurized with acid

Different letters denote significant difference (p<0.05).

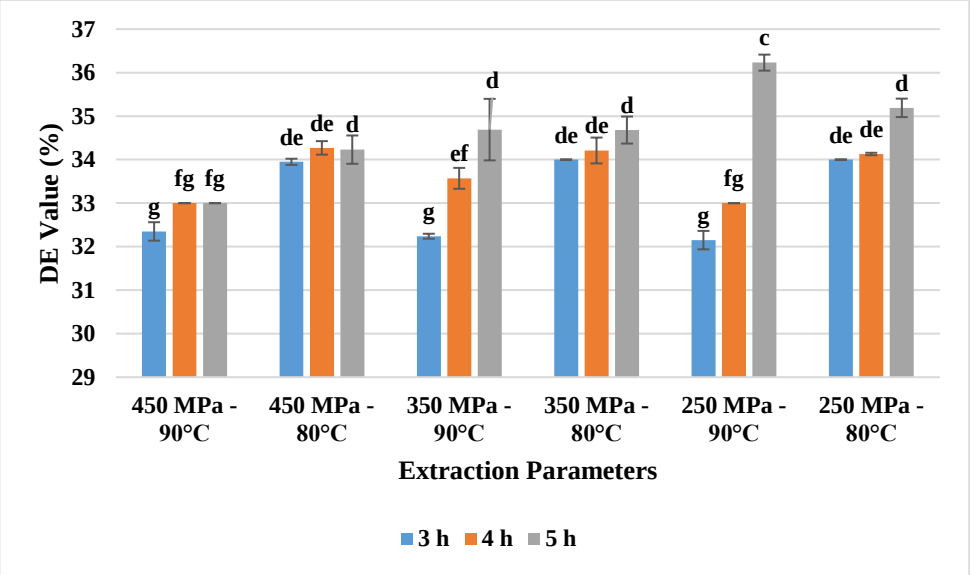

Figure 3. 6. Experimental results of DE Value of pectins extracted by HHP assisted extraction for samples pressurized without acid

Different letters denote significant difference (p<0.05).

According to 1-way ANOVA results, there is no significant difference between 250 and 350 MPa data (p>0.05) where 450 MPa data is significantly different (p<0.05). Temperature data were significantly different from each other and mean of 90°C results were lower in case of DE value (p<0.05). Time data were also significantly different in itself and the lowest mean found at 3 h extractions (p>0.05).

According to 2-way results, pressure did not create significant difference (p>0.05) at 80°C which means that even HHP assisted extraction at 80°C was not enough to affect the molecular structure in great extent. For 90°C, there is no significant difference between 250 and 350 MPa pressure application (p>0.05) while 450 MPa creates significant difference (p<0.05). It is because 250 and 350 MPa pressures are not effective as much as 450 MPa pressure on cell destruction; but at 450 MPa, the pectin released at assistant step and the high destruction in cell wall result in pectin being faced to a high temperature like 90°C through extraction duration and more -OH groups replace with methyl esters. Regarding the pressure-time combinations,

different pressure values did not create significant differences on DE value at 3 h extraction times (p>0.05). 3 h is short enough for both pectin released under high pressure and extracted with high temperature treatment to stay almost unchanged structurally. For samples exposed to different pressures and then to 5 h extraction, the increasing pressure decreased esterification degree because of prolonged time of heat treatment breaking bonds of polymer by causing fractions and the broken part of molecule bonds to -OH group. Regarding the different temperature-time combinations, esterification degree fluctuates between 38.14 ± 0.5 % and 33 ± 1.92 % for samples pressurized with acid and between 36.23 ± 0.18 % and 32.15 ± 0.67 % for samples pressurized without acid. This difference is not enough to be effective on physical and functional properties of pectin such as molecular weight and viscosity (Mesbahi et.al., 2005; Villay et.al., 2012); however, the results of different T-t combinations are significantly different (p<0.05). DE value increases with increasing time but decreases with increasing temperature. By the raising temperature, side chain connections of pectin are destroyed, and pectin become low methoxy pectin and DE value continues to decrease as long as it is exposed to heat. However, during the extraction process, the pectin molecules continue to be released through the all extraction time. In other word, all pectin molecules are not exposed to heat for equal time so regarding the ongoing pectin release, the average DE value tends to increase with time for each extraction.

According to 3-way ANOVA results, considering all P-T-t combinations for both samples pressurized with and without acid, it was seen that temperature was further effective on DE value. As Choo et al. (2013 and) and Venzon et al. (2015) mentioned in their research, the most effective parameter in pectin extraction that accelerate the saponification in pectin structure is temperature and it chemically causes deesterification in side chains of pectin molecule. For both experimental groups, holding the pressurization time 5 min prevent pressure breaking up the side chains of pectin so esterification degree indicated by side chains damaged by heat during extraction gives the final DE. The effect of pressure reveals when prolonged exposure

to heat treatment is considered. Since the pH of the medium to be pressurized for the samples pressurized with acid is low and hydroxyl ion number is low, it is seen that pectins released during pressurization are protected from chemically deesterification at high pressures and the average DE value gives higher results with the effect of increasing yield. For the samples pressurized without acid, high pressures may cause side chain fractions in the released pectin structure during pressurization. Therefore, when samples pressurized with and without acid are compared, it is seen that the samples pressurized with acid indicate higher esterification values, even if there is no big difference between them. All in all, as it is expected 90°C extractions resulted in lowest DE value. For 80°C, as time increases esterification degree also increases by the effect of increasing yield. Since the extraction process cannot be considered as a process in which pectin release continues until a certain point and the pectins obtained in remaining time are exposed to temperature, the continuous pectin release during extraction time acts to increase the mean DE value to be obtained.

3.3. Flow Behavior

Viscosity is one of the most important properties of hydrocolloids in their solutions because these polymers are used for stabilizing solutions and decreasing mobility of solutions by forming gel structure where the viscosity is the main indicator in this case (Guo et al., 2014). In Appendices, Table B.3, Table B.4, Table B.9 and Table B.10 show the apparent viscosities of 2 g/L pectin solutions which were extracted at different conditions. The viscosity differences occur between different pectin solutions may proceed from presence of sucrose, pectin percent in solution, molecular weight of pectin molecules and pH of the solution (Phatak et.al., 1988). In this study, it is thought that the observed differences in viscosity are related with molecular size and so molecular weight because solutions are prepared at the same pH value, without sucrose. Moreover, when taking into consideration that sugar beet pectin has low gelling ability, the viscosity of solution was predicted to be low because gelling ability

and viscosity properties of pectin are interrelated (Phatak et al., 1988). The viscosity results of extracted pectins were found as it was predicted and even, they have Newtonian behavior in 2 g/L concentration as it showed in Figure 3.7.

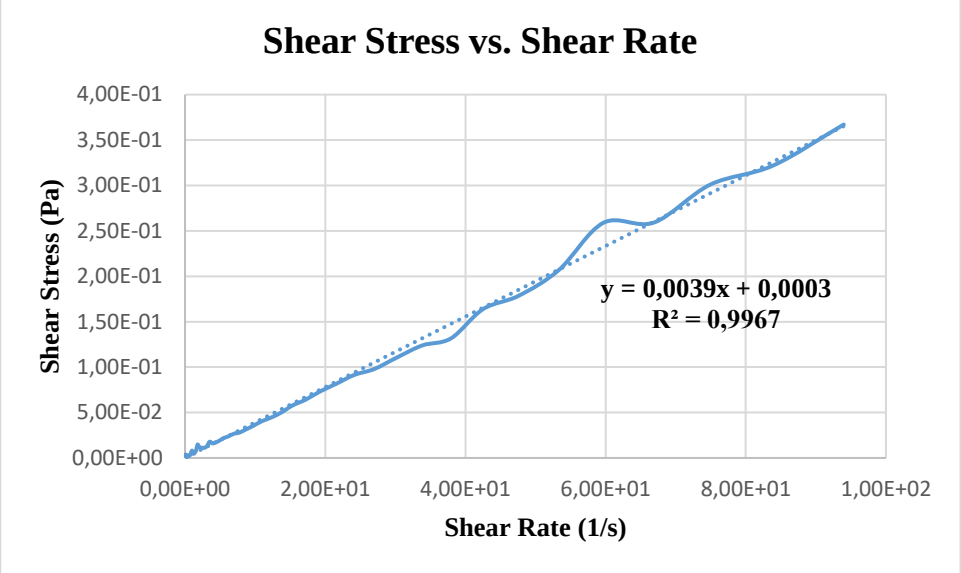

Figure 3. 7. Shear stress vs. shear rate graph of pectin solution which shows the flow behavior of solution in 2 g/L concentration (pectin sample extracted at 350 MPa - 90°C – 4 h and pressurized with acid)

Regarding the conventional extraction results, pressure creates no significant difference (p>0.05) between CE and HHP assisted extraction by itself for both experimental groups. When observing all P-T-t combinations, any results including CE and HHP assisted extraction create significant different, too (p>0.05). So; the effect of HHP to pectin extraction gives stable viscosity results which means improving of extraction process by inserting pressure does not cause question point in pectin quality in terms of viscosity. Although the DE value results were significantly different (p<0.05), the small change between 32-38 % DE value was not reflected on viscosity results and viscosity results showed no significant different (p>0.05) with the exception of samples pressurized with acid results of 450 MPa - 90°C – 3 h, 350 MPa - 90°C – 3 h and 250 MPa - 80°C – 5 h data (p<0.05). The related results for

conventional extraction, and two groups of HHP assisted extraction which are samples pressurized with acid and samples pressurized without acid are given in Figure 3.8, Figure 3.9 and, Figure 3.10, respectively.

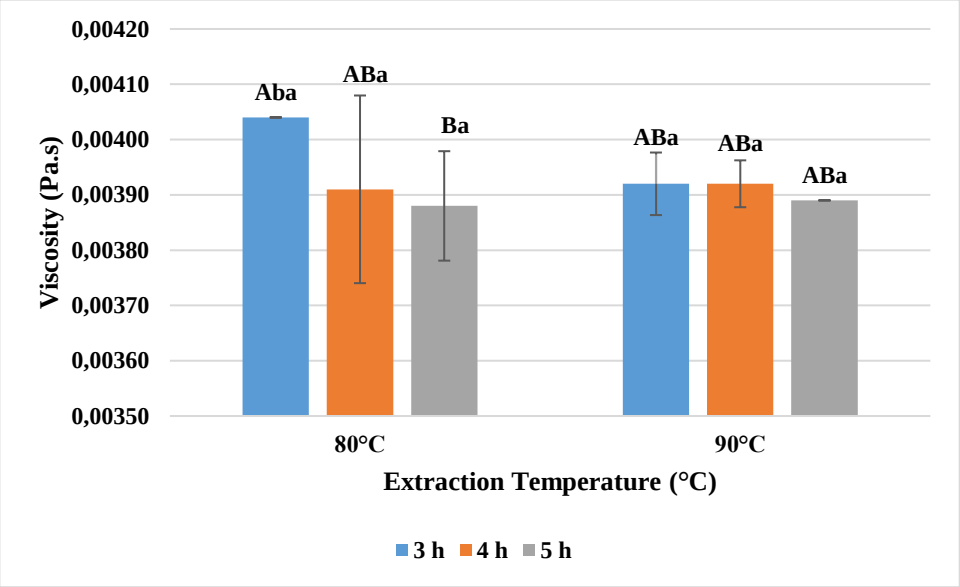

Figure 3. 8. Experimental results of viscosity of pectins extracted by conventional extraction

Different letters denote significant difference (p<0.05). Uppercase letters represent significant difference of samples pressurized with acid while lowercase letters represent statistical analysis of samples pressurized without acid.

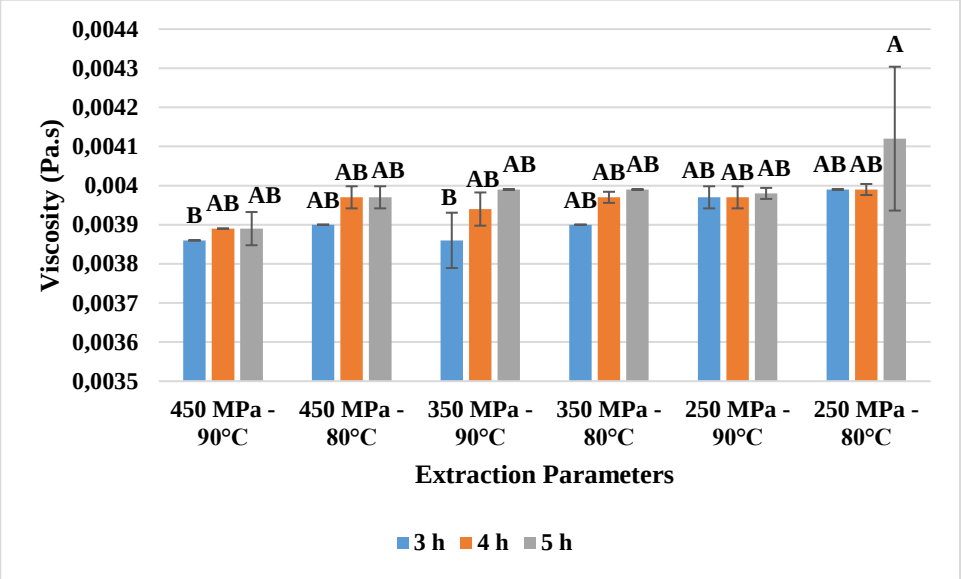

Figure 3. 9. Experimental results of viscosity of pectins extracted by HHP assisted extraction for samples pressurized with acid

Different letters denote significant difference (p<0.05).

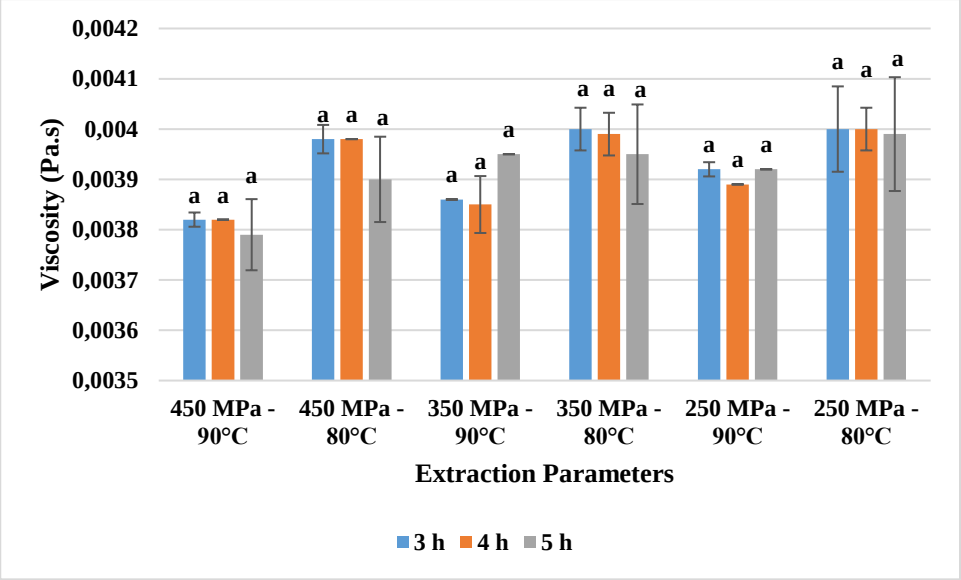

Figure 3. 10. Experimental results of viscosity of pectins extracted by HHP assisted extraction for samples pressurized without acid

Different letters denote significant difference (p<0.05).

According to 1-way ANOVA results, pressure and time data did not show significant difference (p>0.05) where viscosity means of two temperature values are significantly different (p<0.05) but this effect did not reflect in overall result of viscosity when including three parameters. Starting from this point, it can be said that temperature is more critical parameter for viscosity than pressure and time.

According to 2-way ANOVA results, although there is significant difference (p<0.05) between samples extracted at different pressures at 80°C and 90°C, there is no significant difference between samples extracted at the same temperature (p>0.05) even if they treated with different pressures. Moreover, the samples of 80°C showed higher viscosities. Starting from this, it is obvious that temperature is more important than pressure in the case of viscosity and 90°C temperature cause a significant decrease in viscosity (p<0.05). Thus, by considering the primary dependent variable in this study, which is yield, replacing the high temperature by pressure do not only contribute to extraction efficiency but also prevent the undesired changes in characteristics of pectin including viscosity. A distinct difference (p>0.05) was not seen at different pressure-time combinations. Among the different temperature-time combinations, viscosity means of pectin solutions is higher ever at maximum extraction time at 80°C than minimum extraction time of 90°C. On this basis, it was validated that temperature is the most effective variable in handled extraction process.

According to 3-way ANOVA results, it was seen that an increasing effect for viscosity shows up when pectin is extracted at lower temperatures for lower durations by the assistance of high hydrostatic pressure. In consideration of no significant difference (p>0.05) between viscosity values of samples treated with different pressures although they have treated with the same temperature during the same time, the high pressure values which are aimed to reach high extraction yields, do not pose problem for viscosity characteristic of pectin molecules. However, high temperature is problematic for pectin because it destroys molecules and causes pectin degradation which results

information of low molecular weight pectin molecules (Mesbahi et.al., 2005; Bagherian et.al., 2011).

Considering the samples pressurized without acid, it was seen that mean value data were similar, but distribution is different. When all P-T-t combinations are handled, no significant difference was observed in viscosities (p>0.05).

Moreover, the lowest viscosity for samples pressurized without acid was 0,00379 ± 0,00005 Pa.s where it was 0,00389 ± 0,00006 Pa.s for samples pressurized with acid. In summary, addition of acid after high hydrostatic pressure treatment caused lower viscosities. The cell destruction triggered with high pressure makes SBP powder more sensitive to acid and accelerate the pectin release. For samples pressurized without acid, the acid is added directly to extraction medium after pressurization where pectin release is harder than acid added pressurization samples. So, during extraction duration, released pectin molecules are exposed to both acid and high temperature directly and polymer degradation occurs. Molecular weights of degraded molecules decrease, and viscosity also decreases by the effect of both direct acid and temperature for samples pressurized without acid. In other word, this viscosity decrease is higher than samples pressurized with acid. When focusing on the effect of different pressures on viscosity, high pressures caused lower viscosity values. The reason is basically amount of pectin released before heat treatment. At 450 MPa, the released pectin at pressurization step is higher than 350 MPa and 250 MPa. After pressurization, acid added to extraction medium and already extracted pectins face with acid directly for all extraction time and more polymer degradation occurs. Consequently, as pressure increase for samples pressurized without acid, viscosity of pectin decreases; however, for handled parameters, the effect was too weak to reflect in viscosity and create difference.

To sum up; low viscosity values, for that matter Newtonian flow behavior, were obtained in this study as it can be understood from Figure 3.7. However, the low

viscosity characteristic is not a bad quality characteristic for pectin because it is an advantageous case for industry that the pectins which are applicable in variety of area of industry having various characteristics. For instance, pectins showing low viscosities even Newtonian behavior in their solutions have great importance for beverage industry. Especially, for low calorie – high fiber beverages which are gaining popularity in recent years (Sloan, 2018), low viscosity pectins are pretty much preferred.

3.4. Galacturonic Acid (Gal-A) Content

Gal-A content refers galacturonic acid backbone percent of each pectin samples include. This percent is directly related with extraction duration and degradation that pectin molecules faced. In other words, no matter the obtained pectin amount in different P-T-t combinations, the more degradation faced with for each pectin sample indicates the less Gal-A content. However, it was predicted that pressure has indirect effect on Gal-A percent. Because pressure was used in this experimental design only for cell structure destruction or cell membrane damage. As yet extraction process was not started, during pressurization step, pressurization time hold constant as 5 min in order to prevent high amount of pectin release in this step and any effect of pressure to characteristics of already released pectin molecules. Starting from this point, the parameters that degradation is directly related are temperature and time and so it was predicted that Gal-A content results will prove direct effect of these parameters.

Regarding the conventional extraction results, the highest Gal-A content was seen in conventionally extracted pectins and variation of Gal-A content of these pectins was significantly different than HHP assisted ones (p<0.05). Gal-A of 80°C extracted pectins conventionally shows no significant difference with change in time and the same case was valid for 90°C, also (p>0.05). The lowest Gal-A content at pectins of CE was 90°C – 5 h which causes highest molecule degradation. This value was not significantly different than even the lowest HHP assisted extraction parameters (250

MPa – 80°C – 3 h) for both samples pressurized with and without acid (p<0.05). It means that inserting pressure to extraction process triggers the pectin release such a pitch that the easily extracted pectins expose to temperature for much more time and face to break down in galacturonic acid rings of backbone region. However, the decrease was still acceptable for at least 60-65 % Gal-A limitation of FAO for pectin, even the lowest Gal-A values was close to 65 %. The related results for conventional extraction, and two groups of HHP assisted extraction which are samples pressurized with acid and samples pressurized without acid are given in Figure 3.11, Figure 3.12 and, Figure 3.13, respectively.

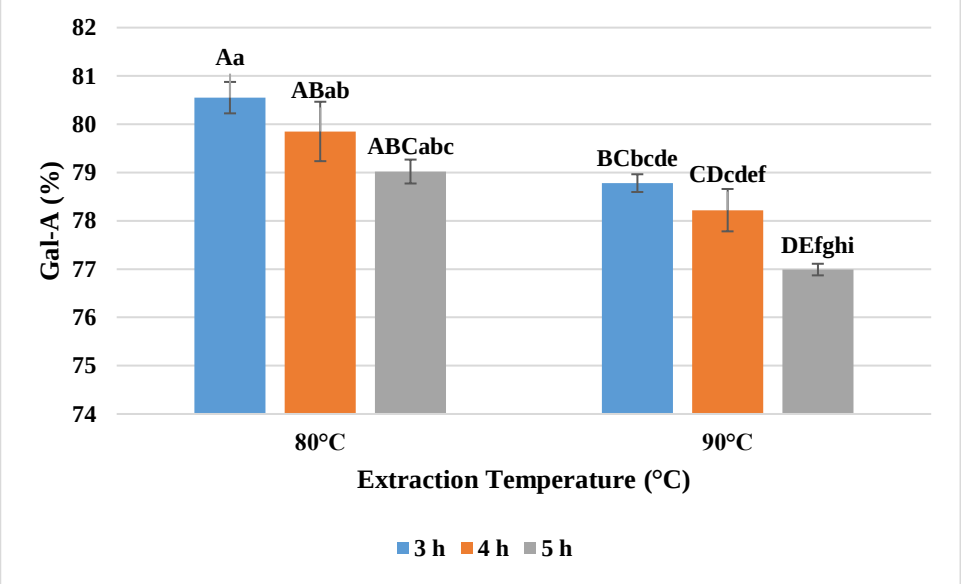

Figure 3. 11. Experimental results of Gal-A content of pectins extracted by conventional extraction

Different letters denote significant difference (p<0.05). Uppercase letters represent significant difference of samples pressurized with acid while lowercase letters represent statistical analysis of samples pressurized without acid.

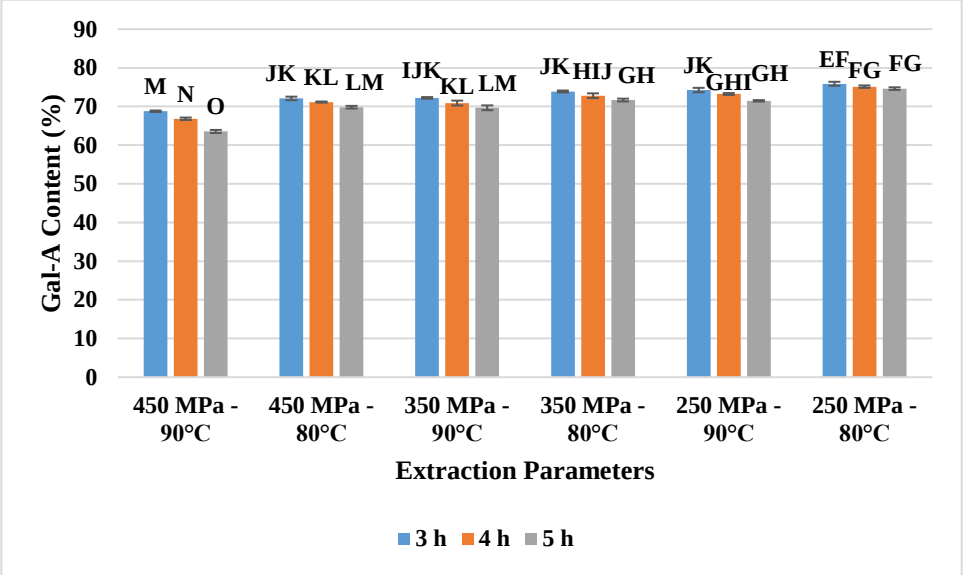

Figure 3. 12. Experimental results of Gal-A content of pectins extracted by HHP assisted extraction for samples pressurized with acid

Different letters denote significant difference (p<0.05).

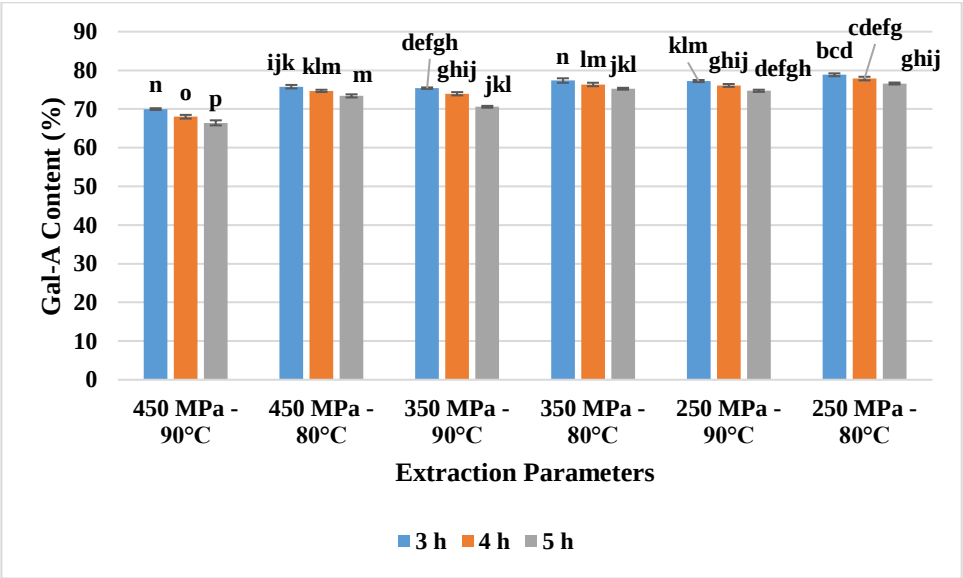

Figure 3. 13. Experimental results of Gal-A content of pectins extracted by HHP assisted extraction for samples pressurized without acid

Different letters denote significant difference (p<0.05). Uppercase letters represent significant difference of samples pressurized with acid while lowercase letters represent statistical analysis of samples pressurized without acid.

According to experimental results, as it was predicted, high temperature and high extraction durations caused high degradations in polymer structure and reduced Gal-A content. It was observed for both samples pressurized with acid and without acid, increasing temperature and time have negative effect on Gal-A percent. The highest Gal-A percent was at lowest T-t combinations, 80°C – 3 h, and at samples pressurized with acid. In order to categorize pectin molecule as functional food additive, FAO requires that it should contain at least 60-65% Gal-A (Food and Agricultural Organization). On the other hand, the pectins obtained in this study was SBP pectins and sugar beet sourced pectin has low gelling ability (Michel et al., 1985) indicates low Gal-A % so the Gal-A results were expected to be low but not lower that 60%. The obtained Gal-A results for samples pressurized with acid were 75.8587 ± 0.37 % maximum and 63.543 ± 0.28 % minimum; for samples pressurized without acid were 78.8569 ± 0.26 % maximum and 66.4327 ± 0.46 % minimum. The results compensated the predictions.

According to 1-way ANOVA results, all of the tree independent variables which are pressure, temperature, time showed significant difference in themselves for both samples pressurized with and without acid ($p<0.05$). In other words, all of each parameter in experimental design changed the characteristic structure and gel making ability of pectin and so Gal-A percent significantly ($p<0.05$). As it is mentioned before, temperature and time have direct effect where pressure have indirect effect on Gal-A %.

According to 2-way ANOVA results, regarding the pressure-temperature data, the lowest Gal-A content was at 450 MPa - 90°C and the highest values were at 250 MPa - 80°C for both experimental groups. These combinations were significantly different from other P-T combinations ($p<0.05$). The reason for that, 250 MPa pressure provides less cell destruction than other two pressures and after pressure application, when extraction duration started a part of this duration was spent to continue to cell destruction. In short, because of pectins are not released rapidly, they faced with heat

less and degraded less than other pressure values. For 450 MPa - 90°C groups, pectin release is triggered even at pressurization step and 90°C cause a high degradation during the extraction at it is mentioned in study of Diaz et al. (2007). Regarding the pressure-time data, for both experimental groups, 250 and 350 MPa data did not show significant difference (p>0.05) where 450 MPa data did (p<0.05) and reached the lowest Gal-A % values. The reason is as it was explained before, pectin molecules which their release highly triggered at 450 MPa, were exposed to heat for longer time. Due to the same reason, 3 and 4 h data showed no significant difference (p>0.05) where 5 h was significantly lower at 250 MPa pressure (p<0.05). The same similarity was observed between 350 MPa – 5 h and 450 MPa – 3 h data. From this point of view, it is understood that keeping the extraction time short and triggering pectin release by assisting the extraction process with increasing pressure not only supports the process efficiency but also backs up extract quality significantly (p<0.05). Addition of acid before or after pressurization did not create difference at action mechanism but pressurization with acid accelerated the pectin degradation and lowered the Gal-A content. Regarding different T-t combinations, considering the degradation factor, the minimum temperature and time were expected to yield maximum Gal-A content for both experimental group and the result was consistent with the literature (Diaz et al., 2007). Between all T-t combinations, the only data are not significantly different were 80°C – 5 h and 90°C – 3 h combinations (p>0.05) which means the Gal-A content of pectins obtained at elevated temperatures can be balanced with pectins obtained at lower temperature by applying the minimum time.

It was predicted that pressure was indirectly effective because of 5 min application duration which is low enough for only triggering cell destruction but not cause high amount of pectin release and pectin degradation at pressurization step. According to 3-way ANOVA results, when the results obtained are examined by considering three parameters, it is seen that degradation can be limited by inserting pressure to the process and decreasing the time. For both samples pressurized with or without acid; there is no significant difference between 250 MPa - 80°C – 5 h, 350 MPa – 80°C – 4

h and 450 MPa – 80°C – 3 h which is a proof of this case (p>0.05). The lowest T-t parameters combined by lowest pressure gives maximum Gal-A content. Thereby, taking into consideration the yield factor descried in the previous parts, replacing the high temperature and time values in production process of pectin will not only increase yield but also significantly contribute to obtain pectin with undestroyed quality characteristics.

3.5. Fourier Transform Infrared Spectroscopy (FTIR) Analysis

The peaks at different spectral regions of pectin that obtained by FTIR spectroscopy was used in characterization of pectin quality and its structural changes after variety of treatments in many researches (Manrique & Lajolo, 2012; Vasco-Correa et al., 2017; Zouambia et al., 2017; Grassino et al., 2016). All of these studies are agreed with that the region can be named as fingerprint of carbohydrate molecules is between 950-1200 cm^{-1} while this region is 1000-2000 cm^{-1} specifically for pectin (Huang et al., 2017). So, the structural changes occur in pectin molecule can be analyzed in detail by observing different spectral regions between 600-3500 cm^{-1} (Cerna et al.,2003). In order to determine the effects of HHP on pectin samples extracted by assistance of HHP, FTIR spectroscopy was used and obtained results were examined by taking into consideration the FTIR analyses of pectin in literature as it is mentioned. All obtained graphs were given below figures including FTIR spectra of standard pectin, conventionally extracted pectins and pectins extracted by HHP assisted method.

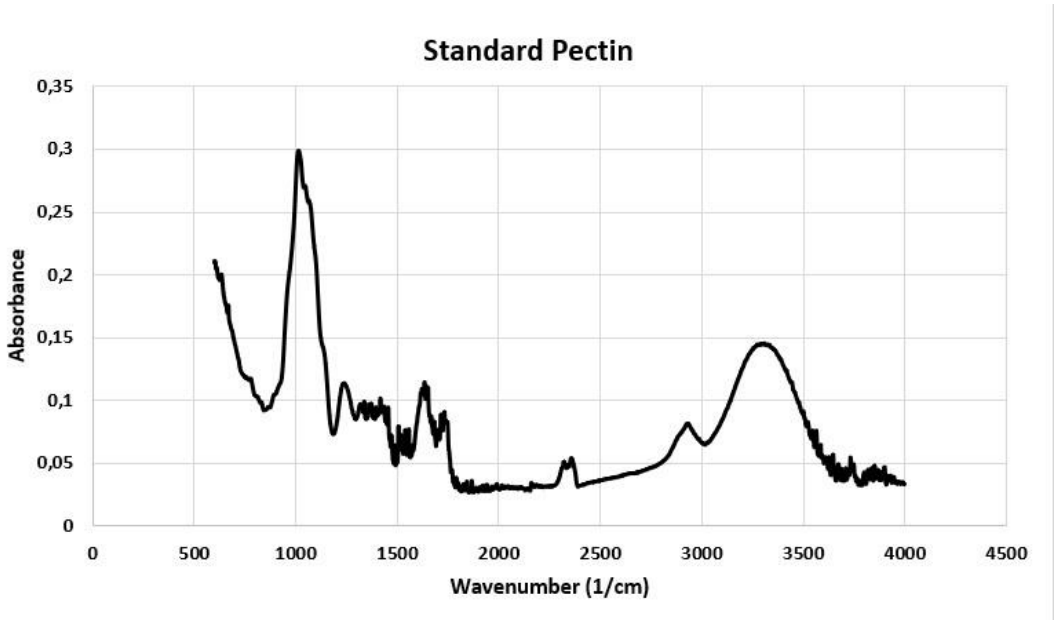

Figure 3. 14. FTIR graph of standard pectin

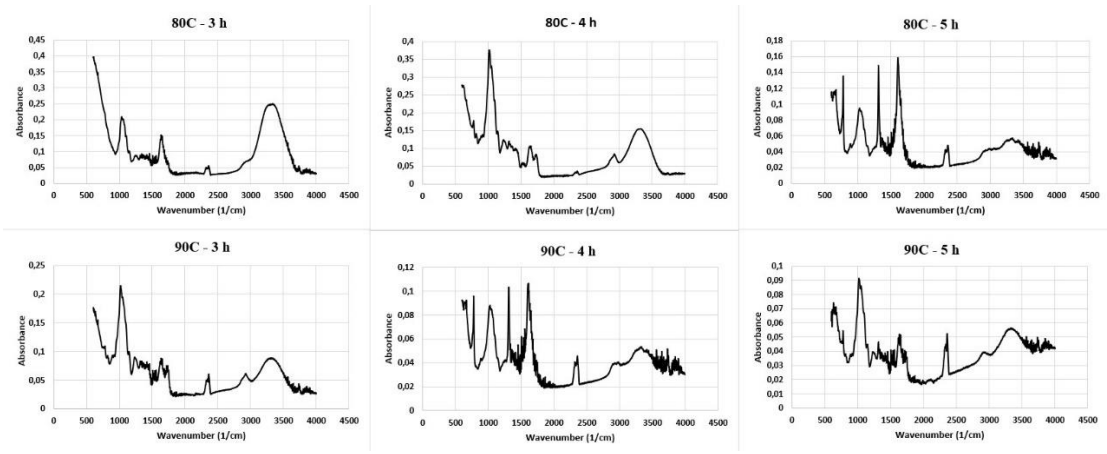

Figure 3. 15. FTIR graph of pectin samples extracted by conventional extraction method

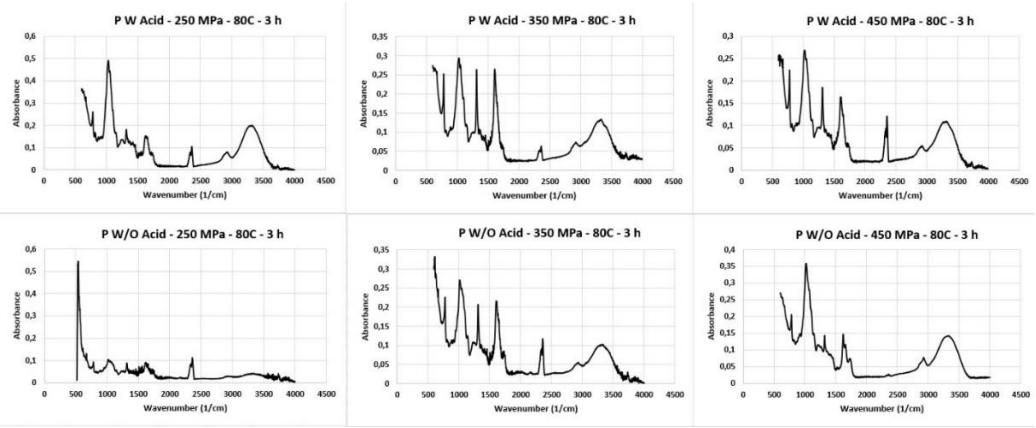

Figure 3. 16. FTIR graph of pectin samples extracted by HHP assisted method at 80°C - 3 h

"P W Acid" and "P W/O Acid" denote samples pressurized with acid and samples pressurized without acid, respectively.

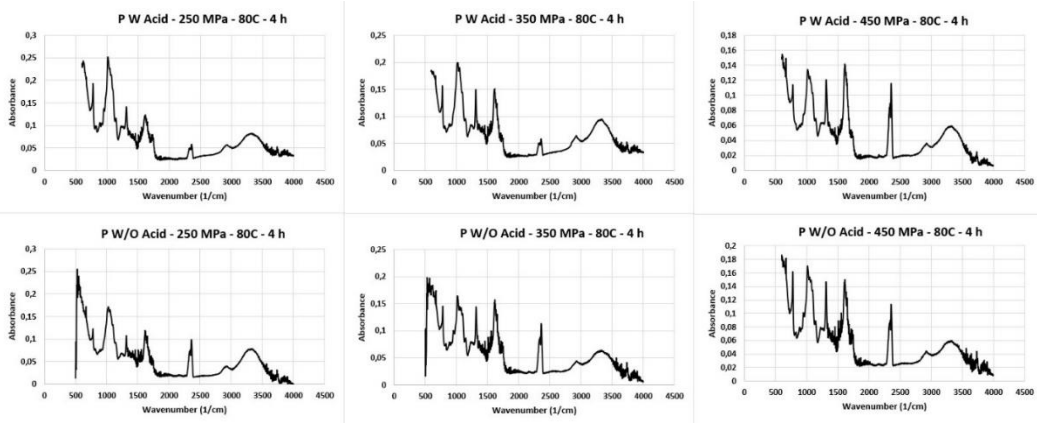

Figure 3. 17. FTIR graph of pectin samples extracted by HHP assisted method at 80°C - 4 h

"P W Acid" and "P W/O Acid" denote samples pressurized with acid and samples pressurized without acid, respectively.

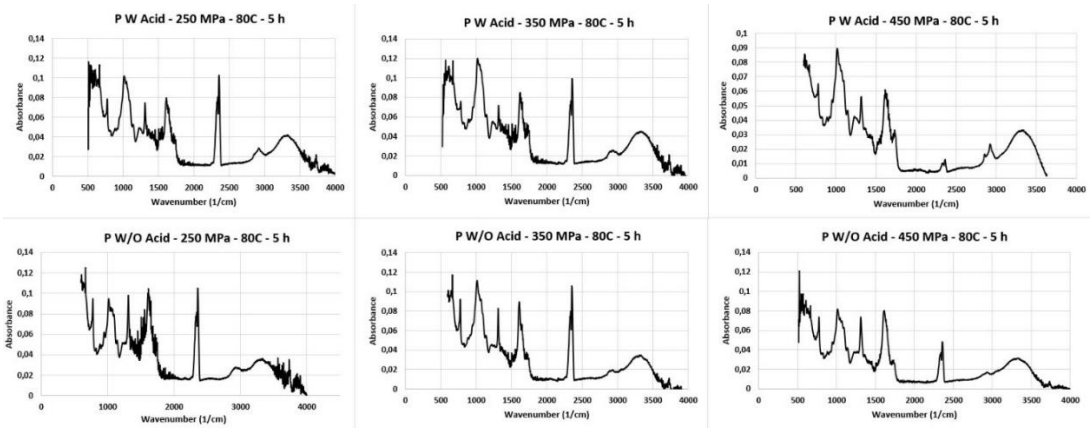

Figure 3. 18. FTIR graph of pectin samples extracted by HHP assisted method at 80°C - 5 h

"P W Acid" and "P W/O Acid" denote samples pressurized with acid and samples pressurized without acid, respectively.

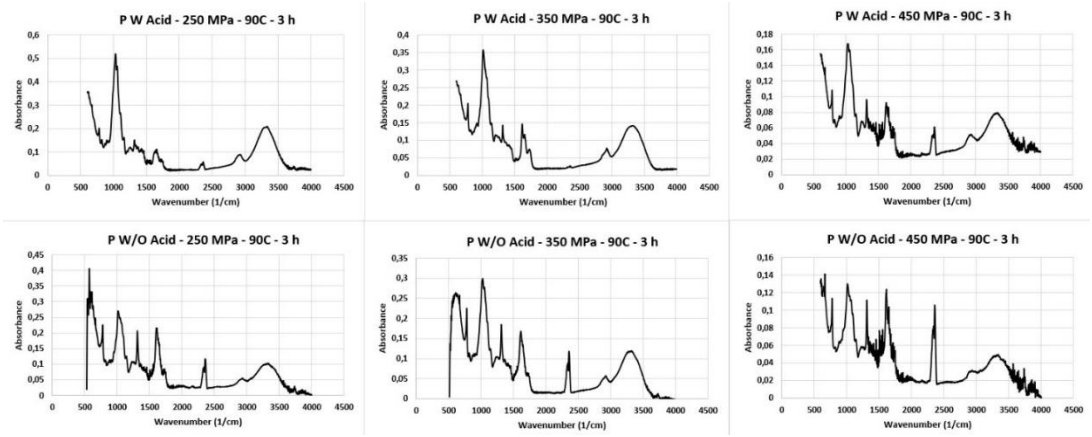

Figure 3. 19. FTIR graph of pectin samples extracted by HHP assisted method at 90°C - 3 h

"P W Acid" and "P W/O Acid" denote samples pressurized with acid and samples pressurized without acid, respectively.

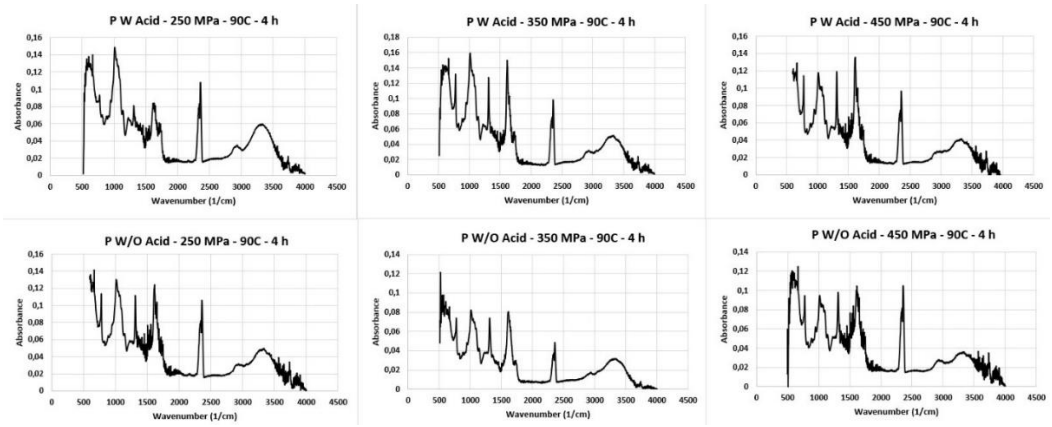

Figure 3. 20. FTIR graph of pectin samples extracted by HHP assisted method at 90°C - 4 h

"P W Acid" and "P W/O Acid" denote samples pressurized with acid and samples pressurized without acid, respectively.

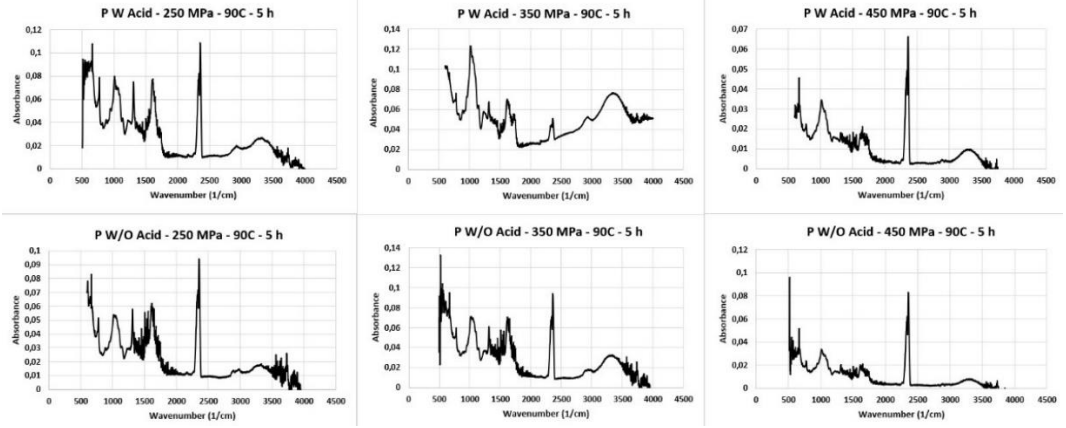

Figure 3. 21. FTIR graph of pectin samples extracted by HHP assisted method at 90°C - 5 h

"P W Acid" and "P W/O Acid" denote samples pressurized with acid and samples pressurized without acid, respectively.

Firstly, the peak at 600-800 cm^{-1} band is examined due to this region indicating the ring structure of pectin that includes C-C bonds and composes skeletal of pectin molecule (Grassino et al., 2016). So, the deformation of these bonds can be related to degradation of pectin molecule. The destruction in skeletal or in backbone ended up with loss of galacturonic acids found in pectin as a result Gal-A content decreased. The height of peaks of 600-800 cm^{-1} band decreased as pressure and temperature increase. The difference at these peaks for the same P-T-t combinations of samples pressurized with acid and samples pressurized without acid was quite low but for samples pressurized without acid the peak heights were higher. This outcome matches up with Gal-A content of pectin samples, as it was expected.

Secondly, the peaks at 1000-2000 cm^{-1} is related with functional properties of pectin so the changes may occur in these peaks have great importance while enhancing the extraction process. According to FTIR analyses of this study, absorbance values of 1000-2000 cm^{-1} peaks were close to each other regardless of acid addition step and pressure level with the exception of samples extracted during 5 hours for both 80°C and 90°C temperature levels. The absorbance values of 5 h extraction samples at 1000-2000 cm^{-1} band were significantly low which indicates the destruction in glycosidic bonds at 1000-1200 cm^{-1} region, in C-H bonds at 1200-1400 cm^{-1} region, in deesterified carboxyl groups at 1400-1600 cm^{-1} region, and in esterified carboxyl groups at 1600-1800 cm^{-1} region (Zouambia et al., 2014).

Another peak seen absorbance graphs of all samples were at near 2400 cm^{-1}. The same peak was seen at research of Huang et al. (2017) at FTIR graphs of pectin samples dried with different methods and stated as unrelated with drying methods because this peak indicates the moisture absorbed by pectin samples.

Moreover, the peak at 2700-3000 cm^{-1} is related with -OH bonds of pectin molecules. The sharp peaks indicate strong -OH bonds at galacturonic rings while board peaks show weak -OH bonds and is correlated with stretched C-H bonds in ring structure of

pectin (Grassino et al., 2016). This peak was obtained at all pectin samples however, it become shorter and boarder as temperature and extraction time increase, especially for 5 h extractions.

Lastly, the peak at 3000-3500 cm^{-1} was analyzed and apparent variations were observed. This peak is significantly board for all pectin samples. 250 and 350 MPa pressure levels did not create interpretable differences at the peak however, the peak was dramatically shorter and boarder at samples pressurized under 450 MPa. Prolonging extraction time also resulted with the same effect with increasing pressure to 450 MPa. Besides, the peak of samples pressurized without acid was boarder, too. This result is thought to be caused by carboxylic acid dimers and hydroxyl groups in pectin molecule making more hydrogen bond and become more stretched at 450 MPa and 5 h conditions and for samples pressurized without acid with respect to other experimental group. It is obvious that the presence of hydrogen ions in extraction environment and the time for extracted pectin molecules to expose these hydrogen ions were result in this peak. In other word, acidic condition and free hydrogen ions are the main reason for the peak at 3000-3500 cm^{-1}. This result coincides with observations of Grassino et al. (2016) that compares the spectral regions of pectins extracted at different extraction conditions. Also, the obtained pectin graphs fitted to FTIR graph of standard industrial pectin which is obtained from Kayseri Şeker.

3.6. Water Holding Capacity (WHC) with Nuclear Magnetic Resonance (NMR) Relaxometry

Regarding conventional extraction (CE) results; the lowest T_2, in other words highest water holding capacity, was obtained at CE by comparing with HHP assisted extraction when the pressure levels are taken into consideration by itself. The T_2 values of 80°C – 3 h and 250 MPa - 80°C – 3 h sows no significant difference for both experimental groups (p>0.05) which means 250 MPa is not enough to change water holding of molecule singly. The same case was valid for 90°C – 5 h and 350 MPa -

90°C – 5h T_2 values (p>0.05); and 90°C – 4 h and 250 MPa - 90°C – 4 h (p>0.05). These results support the betterment of extraction process by inserting HHP because it was seen that the same water holding capacities were reached at the same T-t combinations of both method (CE and HHP assisted method) while inserting to this T-t combination pressure. It was mentioned in previous parts that pressure increases the yield significantly so adding pressure parameter to process hold water holding in certain level while it gives higher yields. As a result, the effectiveness of the pressure assistance was proven for T_2 results, too. The related results for conventional extraction, and two groups of HHP assisted extraction which are samples pressurized with acid and samples pressurized without acid are given in Figure 3.23, Figure 3.24 and, Figure 3.25, respectively. Moreover, the graph that T_2 were read is given in Figure 3.22 as an example which is graph of standard pectin sample.

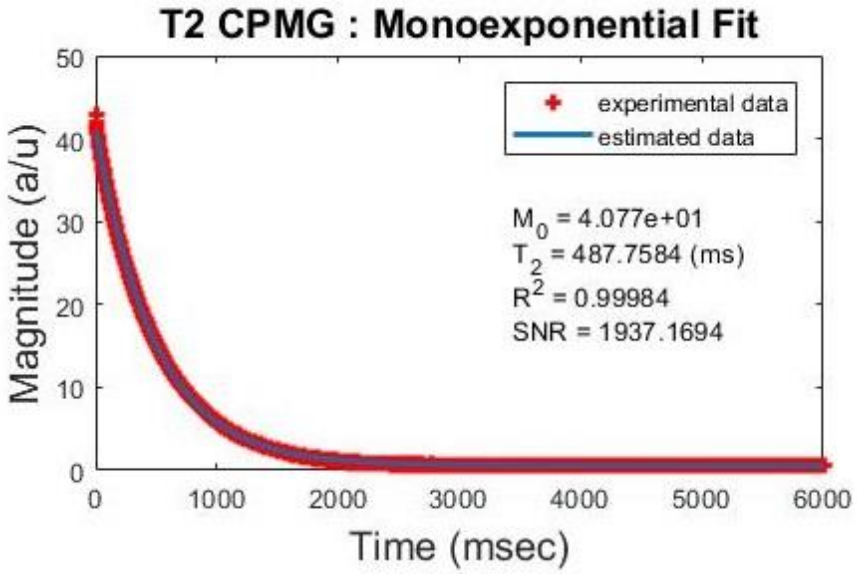

Figure 3. 22. T2 graph of standard pectin obtained by NMR Relaxometry

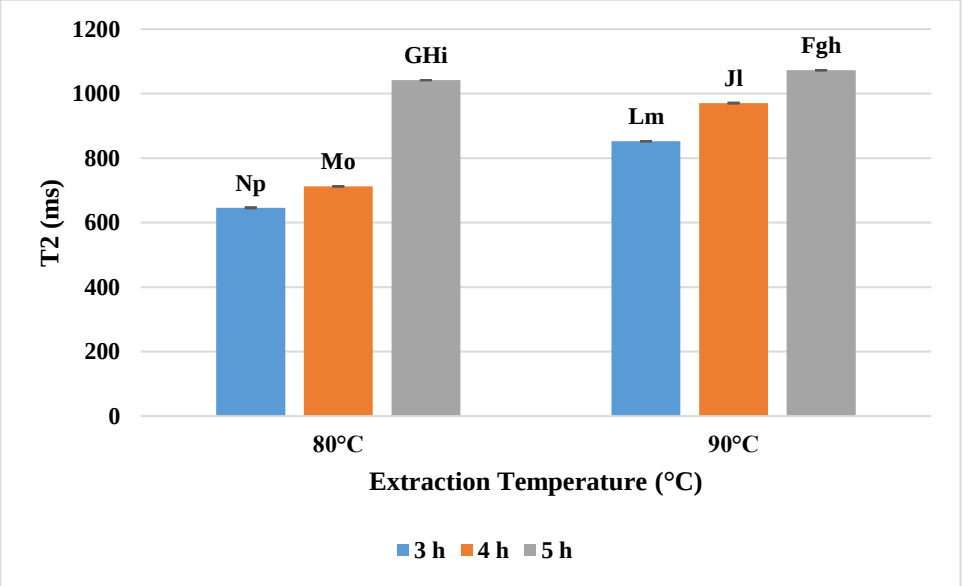

Figure 3. 23. Experimental results of T2 (ms) of pectins extracted by conventional extraction

Different letters denote significant difference (p<0.05). Uppercase letters represent significant difference of samples pressurized with acid while lowercase letters represent statistical analysis of samples pressurized without acid.

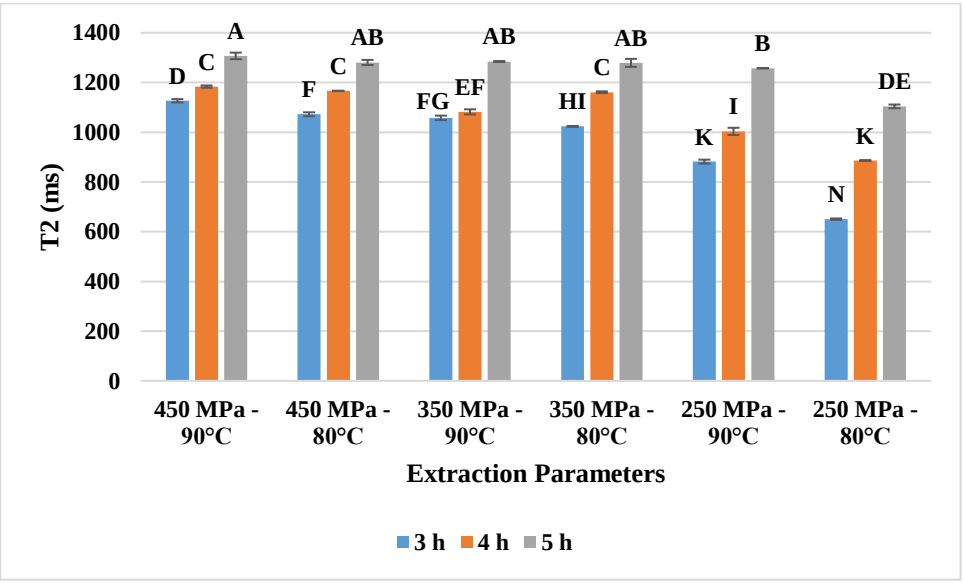

Figure 3. 24. Experimental results of T2 (ms) of pectins extracted by HHP assisted extraction for samples pressurized with acid

Different letters denote significant difference (p<0.05).

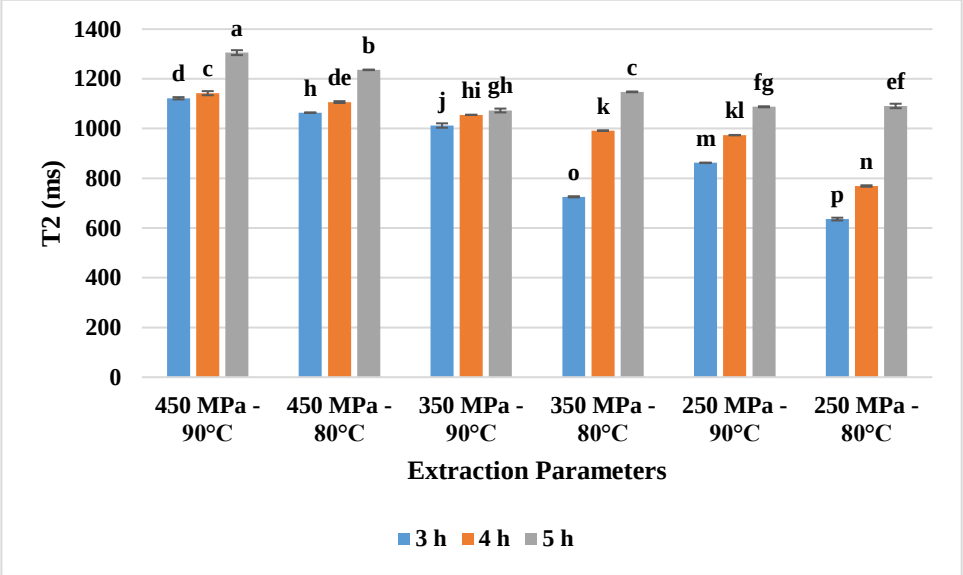

Figure 3. 25. Experimental results of T2 (ms) of pectins extracted by HHP assisted extraction for samples pressurized without acid

Different letters denote significant difference (p<0.05).

According to 1-way ANOVA results, all of the three independent variables which are pressure, temperature, time showed significant difference in themselves for both samples pressurized with and without acid (p<0.05). The highest T_2 values were seen at high pressures, high temperature and long treatment times. So, it can be said that these values have reducing effect on water holding capacity of pectin. T_2 value refers free water and low T_2 values give indication of low free water. In other words, low T_2 values indicates that pectin molecules bind higher amount of water.

According to 2-way results, there is no similarity in results for both experimental group in the case of pressure-temperature combinations (p<0.05). The highest water holding capacity was seen at 250 MPa - 80°C group. Regarding different pressure-time combinations, the results of samples pressurized with acid were not parallel to samples pressurized without acid (p<0.05). For samples pressurized without acid, 450 MPa – 3 h results were similar with 250 MPa – 5 h results (p>0.05) and 250 MPa – 4 h results were close to 350 MPa – 3 h results (p>0.05). In the previous discussions,

including the high hydrostatic pressure to extraction process was found as promoter for extraction yield. Also, for NMR results, the increasing pressure replaced the long extraction times and keep the water holding capacity as much as high at 250 MPa – 4 h. For samples pressurized with acid, 250 MPa – 5 h results were close to 450 MPa – 4 h results (p>0.05) and 450 MPa – 5 h results were close to 350 MPa – 5 h results (p>0.05). However, as it is said before, high pressures and high extraction durations lowered the water holding capacity. So, similarities of highest pressure levels and longest extraction times were not influential for improvement of extraction by high hydrostatic pressure.

By analyzing 3-way ANOVA results, the effect of three independent variables on water holding capacity of pectin molecules became more meaningful. For samples pressurized without acid, 350 MPa - 80°C – 4 h results and 250 MPa - 90°C – 4 h results were not significantly different (p>0.05). This situation shows increasing the pressure while taking the time constant gives the same water holding capacity even at lower temperatures. Even if temperature was raised to 90°C, the same water holding capacity with 250 MPa was obtained by applying 3 hours extraction. More similar results were obtained as an evidence of this situation which can be seen at Appendices Table B.14. For samples pressurized with acid, 250 MPa - 90°C – 4 h results were not significantly different than 350 MPa - 80°C – 3 h results (p>0.05) which means that close WHC can be attained at lower temperatures and lower extraction times by increasing the pressure. Considering that high pressures point out increasing efficiencies and are found effective to enhance the process in this study, pressure was considered as having positive effect on maintenance of water holding capacity, too. Invariably, more similarity results were determined in between different P-T-t combinations which proves the mentioned case (Appendices, Table B.13).

All in all, all findings were commentated and combined. Water holding capacity of pectin is dependent on available hydrophilic groups of pectin. When remembering the esterification mechanism, pectin was including -OH groups that are replaced by

methyl groups during esterification reactions. So, the esterification degree is one of the important factors for water holding capacity of pectin. Regarding the deesterification, more hydrophilic groups on pectin are available so water holding ability is higher. Except that, bulk density of pectin, its galacturonic acid content and extraction conditions also have great importance on water holding capacity. Water holding capacity of pectins extracted at different conditions are compared by considering these factors. Pectins extracted by the method of pressurization without acid hold more water with respect to method of pressurization with acid because they have lower esterification degrees, firstly. They can make bond with more water molecules thanks to their -OH groups on carboxylic acid groups. Moreover, samples pressurized without acid have higher galacturonic acid groups. The viscosity results were insignificant for experimental group (p<0.05) so they were not included when commenting on water holding capacity. At different temperatures; esterification degree is lower at 90°C but viscosity results are incomparably close to each other while Gal-A contents are higher at 80°C. That is to say, the T_2 results were higher at 90°C than 80°C as expected. At different extraction times, esterification degrees are increase with time while Gal-A content decreased due to degradation of pectin molecules. By taking into consideration the increasing treatments at prolonging times, extrinsic factors are included in balance for water holding capacity and these factors results in an increase for T_2 values. Consequently, increasing time causes a fall in water holding capacity.

CHAPTER 4

CONCLUSION AND RECOMMENDATIONS

In this study, the effect of HHP assisted extraction on improvement of pectin extraction and on quality characteristics of extracted pectin were discovered. Conventional extraction (80°C and 90°C; 3, 4 and 5 h) results and findings were compared with results of HHP assisted method which pressurization step conducted with or without addition of acid, different pressure levels (250, 350 and 450 MPa), extraction temperature variations (80°C and 90°C) and different extraction times (3, 4 and 5 h). In order to prevent significant amount of pectin release during pressurization step, the pressurization was applied for only 5 min at 40°C.

The experimental results revealed that HHP treatment of SBP before starting the extraction procedure was definitely effective on increasing the extraction yield regarding the use of the same temperature-time combination at both conventional extraction and HHP assisted extraction, i.e. 6.43 ± 0.07 % yield at 90°C – 5 h combination of CE and almost doubled 12.23 ± 0.13 % yield at 450 MPa - 90°C – 5 h extraction of samples pressurized with acid. However, by taking into consideration the quality characteristics of extracted pectins, the optimum extraction condition differed based on the pectin properties intended to be obtained i.e. for low DE value pectin, 32.35 ± 0.15 % DE value at 450 MPa - 90°C – 3 h extraction parameters. According to the experimental results, effect of HHP on both samples pressurized with and without acid was significant (p<0.05) but the HHP applied on acidified SBP medium was higher. This means the destruction of cell wall was triggered in a considerable extend and pectin release was eased with pressure assistance while acid and pressure showed synergistic effect on cell wall distortion.

On the other hand, esterification degree of extracted pectins decreased with employing HHP because of pectins being more easily released and faced with high temperatures through extraction time. Acidic extraction conditions also decreased DE value due to formation of fractions in pectin structure by -H and -OH ion interaction of pectin molecule from environment. This effect was higher for samples pressurized without acid because the acid added after pressurization was included in an environment that already have a fair amount of pectin while the being released pectins in extraction also face with acid, too. Pectin extracted at 450 MPa - 90°C – 5 h with acid had 33.5 ± 0.15 % DE while pectin extracted at 450 MPa - 90°C – 5 h without acid had 33 ± 0.0 %. DE value of conventionally extracted pectin samples was close to HHP assisted extracted pectins at the same temperature-time at 250 MPa (p<0.05). However, pressurization created decreasing DE values at prolonged extraction times. Regarding overall DE values were between 32-38%, the decrease found low enough to not reflect in viscosity of pectin solutions and change in viscosity was insignificant (p>0.05) at almost all P-T-t combinations while viscosity insignificantly (p<0.05) decreased with respect to conventional extraction.

Also, HHP was decreased the Gal-A content of pectin by fasten the extraction and let pectins face temperature for longer times which result in polymer degradation. However, this decrease was still in the safe limits to meet the FAO requirements for pectin to have 60-65 % Gal-A content, i.e. Gal-A content for samples pressurized without acid at 450 MPa - 90°C – 5 h was 66.43 ± 0.46 %.

In this study, NMR experiments were also conducted, and it was found that water holding capacity of pectin molecules decrease with increasing pressure and temperature because of the combined effect of change in esterification degree, galacturonic acid, viscosity and extending treatments as an extrinsic factor. For samples pressurized without acid water holding capacity is higher because of DE value

being lower and presence of more -OH groups on carboxylic acid groups to make bonds with water molecules while their Gal-A contents and viscosities were insignificantly different (p>0.05). For different temperatures, viscosity values were incomparably close to each other (p>0.05), but Gal-A content were lower at higher temperature because of polymer degradation; so, the water holding capacity was lower. As comparing with conventional extraction, water holding capacities were decreased in pectins of HHP assisted method because of the same reasons as follows: T_2 of pectin extracted conventionally at 80°C – 3 h was 646.0148 ± 1.3565 ms while it was 725.5123 ± 1.619 ms at 350 MPa – 80°C – 3h and 1063.843 ± 0.567 ms at 450 MPa – 80°C – 3h for samples pressurized without acid.

To sum up, inserting high hydrostatic pressure to extraction process found effective on increasing the pectin extraction yield even with decreased process parameters while it resulted in variations of functional properties of pectin. However, these changes were considered as a chance to meet the needs of different areas of food industry by obtaining pectins with different properties by changing extraction parameters. This is because different areas in industry needs pectins with different characteristic like low methoxylated or high methoxylated pectins and low gelling ability or high gelling ability pectin. The pectins extracted in this study can be an option for beverage industry, especially for low calorie – high fiber beverage production and for syrup production of pharmaceutical industry by the advantage of showing low viscosities even Newtonian flow behavior in their solution with low concentrations. For example, the pectin samples extracted at 350 MPa - 90°C – 3 h as pressurized with acid was one of the best options for recommending to mentioned industries. Moreover, this study proved that HHP is an influential and advantageous method for food waste utilization and obtaining functional ingredients from these wastes. This method may be employed for valorization of many functional ingredients other than pectin from many sources like polysaccharides, phenolics, anthocyanins, proteins and other high-added value compounds.

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APPENDICES

A. Calibration Curve

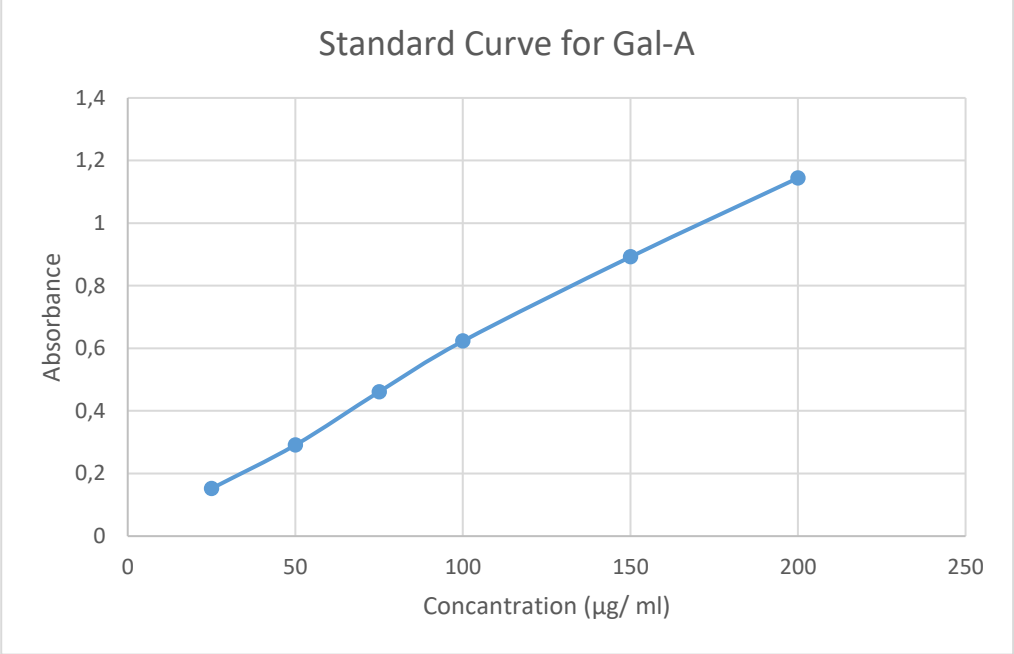

Figure A. 1. Calibration curve for determination of galacturonic acid content

Absorbance (at 520 nm) = 0.0057 * (µg Gal-A/ml) + 0.022 (Eq. 3)

B. Comparative Tables

Table B. 1. Experimental results of pectin yield, DE value and Gal-A content of pectins extracted by conventional extraction and compared with samples pressurized with acid

Extraction Parameters		Pectin Yield (%)	DE Value (%)	Gal-A (%)
Temperature (°C)	Time (h)			
90	5	6.43 ± 0.11^{H}	37.28 ± 0.04^{ABCD}	76.99 ± 0.09^{DE}
	4	6.26 ± 0.22^{H}	34.05 ± 0.03^{FGHI}	78.22 ± 0.31^{CD}
	3	3.37 ± 0.08^{KL}	34.0 ± 0.0^{GHI}	78.78 ± 0.13^{BC}
80	5	5.68 ± 0.21^{IJ}	39.18 ± 0.05^{A}	79.02 ± 0.18^{ABC}
	4	5.11 ± 0.36^{J}	37.90 ± 0.10^{ABC}	79.85 ± 0.44^{AB}
	3	3.02 ± 0.13^{L}	36.03 ± 0.02^{CDEF}	80.55 ± 0.23^{A}

Different letters denote significant difference ($p<0.05$).

Table B. 2. Experimental results of pectin yield, DE value and Gal-A content of pectins extracted by conventional extraction and compared with samples pressurized without acid

Extraction Parameters		Pectin Yield (%)	DE Value (%)	Gal-A (%)
Temperature (°C)	Time (h)			
90	5	6.43 ± 0.11^{hij}	37.28 ± 0.04^{b}	76.99 ± 0.09^{fghi}
	4	6.26 ± 0.22^{ijk}	34.05 ± 0.03^{de}	78.22 ± 0.31^{cdef}
	3	3.37 ± 0.08^{mn}	34.0 ± 0.0^{de}	78.78 ± 0.13^{bcde}
80	5	5.68 ± 0.21^{kl}	39.18 ± 0.05^{a}	79.02 ± 0.18^{abc}
	4	5.11 ± 0.36^{l}	37.90 ± 0.10^{b}	79.85 ± 0.44^{ab}
	3	3.02 ± 0.13^{n}	36.03 ± 0.02^{c}	80.55 ± 0.23^{a}

Different letters denote significant difference ($p<0.05$).

Table B. 3. Experimental results of viscosity and T2 value of pectins extracted by conventional extraction and compared with samples pressurized with acid

Extraction Parameters		Viscosity (Pa.s)	T_2 (ms)
Temperature (°C)	Time (h)		
90	5	0.00389 ± 0.0000^{A}	1072.2599 ± 0.509^{F}
	4	0.00392 ± 0.00003^{AB}	970.6262 ± 1.1745^{J}
	3	0.00392 ± 0.00004^{AB}	852.1167 ± 0.494^{L}
80	5	0.00388 ± 0.0001^{B}	1041.6041 ± 0.489^{GH}
	4	0.00391 ± 0.00013^{AB}	712.2684 ± 0.4835^{M}
	3	0.00404 ± 0.0000^{AB}	646.0148 ± 1.3565^{N}

Different letters denote significant difference ($p<0.05$).

Table B. 4. Experimental results of viscosity and T2 value of pectins extracted by conventional extraction and compared with samples pressurized without acid

Extraction Parameters		Viscosity (Pa.s)	T_2 (ms)
Temperature (°C)	Time (h)		
90	5	0.00389 ± 0.0000^{a}	1072.2599 ± 0.509^{gh}
	4	0.00392 ± 0.00003^{a}	970.6262 ± 1.1745^{l}
	3	0.00392 ± 0.00004^{a}	852.1167 ± 0.494^{m}
80	5	0.00388 ± 0.0001^{a}	1041.6041 ± 0.489^{i}
	4	0.00391 ± 0.00013^{a}	712.2684 ± 0.4835^{o}
	3	0.00404 ± 0.0000^{a}	646.0148 ± 1.3565^{p}

Different letters denote significant difference ($p<0.05$).

Table B. 5. Experimental results of pectin yield for samples pressurized with acid

Pressure (MPa)	Extraction Parameters		Pectin Yield (%)
	Temperature (°C)	Time (h)	
450	90	5	12.23 ± 0.13^{A}
		4	10.86 ± 0.09^{B}
		3	9.34 ± 0.03^{C}
	80	5	10.23 ± 0.12^{B}
		4	8.39 ± 0.05^{DE}
		3	7.47 ± 0.04^{FG}
350	90	5	9.48 ± 0.06^{C}
		4	9.03 ± 0.05^{CD}
		3	7.35 ± 0.03^{FG}
	80	5	7.42 ± 0.04^{FG}
		4	7.26 ± 0.02^{G}
		3	6.45 ± 0.01^{H}
250	90	5	7.93 ± 0.04^{EF}
		4	7.14 ± 0.04^{G}
		3	3.91 ± 0.02^{K}
	80	5	7.14 ± 0.04^{G}
		4	5.74 ± 0.02^{IJ}
		3	3.71 ± 0.01^{K}

Different letters denote significant difference ($p<0.05$).

Table B. 6. Experimental results of pectin yield for samples pressurized without acid

Pressure (MPa)	Extraction Parameter		Pectin Yield (%)
	Temperature (°C)	Time (h)	
450	90	5	12.09 ± 0.11^{a}
		4	9.45 ± 0.10^{b}
		3	8.77 ± 0.09^{c}
	80	5	8.54 ± 0.10^{cd}
		4	7.96 ± 0.04^{de}
		3	7.02 ± 0.03^{fgh}
350	90	5	8.99 ± 0.05^{bc}
		4	8.35 ± 0.06^{cd}
		3	7.35 ± 0.05^{efg}
	80	5	7.16 ± 0.04^{fg}
		4	6.89 ± 0.07^{fghi}
		3	6.39 ± 0.02^{hij}
250	90	5	7.52 ± 0.04^{ef}
		4	6.76 ± 0.04^{ghi}
		3	3.68 ± 0.01^{m}
	80	5	5.89 ± 0.02^{jk}
		4	5.21 ± 0.04^{l}
		3	3.47 ± 0.02^{mn}

Different letters denote significant difference ($p<0.05$).

Table B. 7. Experimental results of DE Values for samples pressurized with acid

Pressure (MPa)	Extraction Parameters		DE Value (%)
	Temperature (°C)	Time (h)	
450	90	5	33.5 ± 0.20^{HI}
		4	33.2 ± 0.20^{I}
		3	33.0 ± 1.92^{I}
	80	5	38.0 ± 1.01^{ABC}
		4	36.0 ± 0.00^{CDEFG}
		3	35.0 ± 0.00^{EFGHI}
350	90	5	35.0 ± 0.00^{EFGHI}
		4	33.6 ± 0.20^{HI}
		3	33.7 ± 0.10^{HI}
	80	5	35.0 ± 0.00^{AB}
		4	33.6 ± 0.20^{BCDE}
		3	33.7 ± 0.05^{DEFGH}
250	90	5	37.2 ± 0.30^{ABCD}
		4	37.0 ± 0.00^{BCDE}
		3	34.0 ± 0.00^{GHI}
	80	5	37.5 ± 0.50^{ABCD}
		4	38.2 ± 0.12^{AB}
		3	36.0 ± 0.00^{CDEFG}

Different letters denote significant difference ($p<0.05$).

Table B. 8. Experimental results of DE Values for samples pressurized without acid

Pressure (MPa)	Extraction Parameter		DE Value (%)
	Temperature (°C)	Time (h)	
450	90	5	33.00 ± 0.00^{fg}
		4	33.00 ± 0.00^{fg}
		3	32.35 ± 0.15^{g}
	80	5	34.23 ± 0.27^{d}
		4	34.27 ± 0.11^{de}
		3	33.95 ± 0.05^{de}
350	90	5	34.69 ± 1.50^{d}
		4	33.57 ± 0.17^{ef}
		3	32.24 ± 0.04^{g}
	80	5	34.68 ± 0.22^{d}
		4	34.21 ± 0.21^{de}
		3	34.00 ± 0.00^{de}
250	90	5	36.23 ± 0.18^{c}
		4	33.00 ± 0.00^{fg}
		3	32.15 ± 0.15^{g}
	80	5	35.19 ± 0.67^{d}
		4	34.13 ± 0.02^{de}
		3	34.00 ± 0.00^{de}

Different letters denote significant difference ($p<0.05$).

Table B. 9. Experimental results of viscosity for samples pressurized with acid

Pressure (MPa)	Extraction Parameters		Viscosity (Pa.s)
	Temperature (°C)	Time (h)	
450	90	5	0.00389 ± 0.00006^{AB}
		4	0.00389 ± 0.00000^{AB}
		3	0.00386 ± 0.00000^{B}
	80	5	0.00397 ± 0.00004^{AB}
		4	0.00397 ± 0.00004^{AB}
		3	0.00390 ± 0.00000^{AB}
350	90	5	0.00399 ± 0.00000^{AB}
		4	0.00394 ± 0.00006^{AB}
		3	0.00386 ± 0.00001^{B}
	80	5	0.00399 ± 0.00000^{AB}
		4	0.00397 ± 0.00002^{AB}
		3	0.00390 ± 0.00000^{AB}
250	90	5	0.00398 ± 0.00002^{AB}
		4	0.00397 ± 0.00004^{AB}
		3	0.00397 ± 0.00004^{AB}
	80	5	0.00412 ± 0.00026^{A}
		4	0.00399 ± 0.00002^{AB}
		3	0.00399 ± 0.00000^{AB}

Different letters denote significant difference ($p<0.05$).

Table B. 10. Experimental results of viscosity for samples pressurized without acid

Pressure (MPa)	Extraction Parameter		Viscosity (Pa.s)
	Temperature (°C)	Time (h)	
450	90	5	0.00379 ± 0.00005[a]
		4	0.00382 ± 0.00000[a]
		3	0.00382 ± 0.00001[a]
	80	5	0.00390 ± 0.00006[a]
		4	0.00398 ± 0.00000[a]
		3	0.00398 ± 0.00002[a]
350	90	5	0.00395 ± 0.00000[a]
		4	0.00385 ± 0.00004[a]
		3	0.00386 ± 0.00000[a]
	80	5	0.00395 ± 0.00007[a]
		4	0.00399 ± 0.00002[a]
		3	0.00400 ± 0.00003[a]
250	90	5	0.00389 ± 0.00000[a]
		4	0.00389 ± 0.00000[a]
		3	0.00392 ± 0.00001[a]
	80	5	0.00399 ± 0.00008[a]
		4	0.00400 ± 0.00003[a]
		3	0.00400 ± 0.00006[a]

Different letters denote significant difference ($p<0.05$).

Table B. 11. Experimental results of galacturonic acid for samples pressurized with acid

Pressure (MPa)	Extraction Parameters		Gal-A (%)
	Temperature (°C)	Time (h)	
450	90	5	63.54 ± 0.28^{O}
		4	66.82 ± 0.22^{N}
		3	68.76 ± 0.14^{M}
	80	5	69.81 ± 0.24^{LM}
		4	71.14 ± 0.10^{KL}
		3	72.08 ± 0.32^{JK}
350	90	5	69.66 ± 0.44^{LM}
		4	70.88 ± 0.45^{KL}
		3	72.21 ± 0.15^{IJK}
	80	5	71.66 ± 0.26^{JK}
		4	72.78 ± 0.43^{HIJ}
		3	73.87 ± 0.18^{GH}
250	90	5	71.46 ± 0.13^{JK}
		4	73.72 ± 0.17^{GHI}
		3	74.24 ± 0.41^{GH}
	80	5	74.62 ± 0.24^{FG}
		4	75.12 ± 0.22^{FG}
		3	75.86 ± 0.37^{EF}

Different letters denote significant difference ($p<0.05$).

Table B. 12. Experimental results of galacturonic acid for samples pressurized without acid

Pressure (MPa)	Extraction Parameter		Gal-A (%)
	Temperature (°C)	Time (h)	
450	90	5	66.43 ± 0.46^{p}
		4	68.01 ± 0.34^{o}
		3	69.99 ± 0.16^{n}
	80	5	73.40 ± 0.29^{m}
		4	74.69 ± 0.21^{klm}
		3	75.78 ± 0.32^{ijk}
350	90	5	70.58 ± 0.17^{n}
		4	73.95 ± 0.30^{lm}
		3	75.39 ± 0.14^{jkl}
	80	5	75.25 ± 0.17^{jkl}
		4	76.35 ± 0.32^{ghij}
		3	77.37 ± 0.41^{defgh}
250	90	5	74.72 ± 0.20^{klm}
		4	76.10 ± 0.26^{hijk}
		3	77.24 ± 0.19^{efghi}
	80	5	76.60 ± 0.18^{ghij}
		4	77.88 ± 0.33^{cdefg}
		3	78.86 ± 0.26^{bcd}

Different letters denote significant difference ($p<0.05$).

Table B. 13. Experimental results of T2 for samples pressurized with acid

Pressure (MPa)	Extraction Parameter		T_2 (ms)
	Temperature (°C)	Time (h)	
450	90	5	1306.9766 ± 0.109^{A}
		4	1183.4299 ± 3.209^{C}
		3	1126.5362 ± 4.711^{D}
	80	5	1280.5206 ± 7.263^{AB}
		4	1166.1317 ± 0.636^{C}
		3	1072.1717 ± 5.592^{F}
350	90	5	1284.3451 ± 1.271^{AB}
		4	1081.8599 ± 7.256^{EF}
		3	1057.7697 ± 6.378^{FG}
	80	5	1278.923 ± 11.316^{AB}
		4	1160.7541 ± 2.495^{C}
		3	1160.7541 ± 2.495^{HI}
250	90	5	1257.3278 ± 0.602^{B}
		4	1003.3769 ± 10.203^{I}
		3	881.8839 ± 5.549^{K}
	80	5	1103.9332 ± 5.283^{DE}
		4	886.0863 ± 0.911^{K}
		3	650.4621 ± 1.897^{N}

Different letters denote significant difference ($p<0.05$).

Table B. 14. Experimental results of T2 for samples pressurized without acid

Pressure (MPa)	Extraction Parameter		T_2 (ms)
	Temperature (°C)	Time (h)	
450	90	5	1305.3114 ± 7.036^{a}
		4	1142.2754 ± 5.825^{c}
		3	1121.7546 ± 3.282^{d}
	80	5	1236.0851 ± 0.864^{b}
		4	1106.5506 ± 2.554^{de}
		3	1063.8432 ± 0.567^{h}
350	90	5	1072.6304 ± 5.472^{gh}
		4	1055.2071 ± 0.138^{hi}
		3	1012.1814 ± 6.231^{j}
	80	5	1147.6152 ± 1.109^{c}
		4	991.6493 ± 0.762^{k}
		3	725.5123 ± 1.619^{o}
250	90	5	1087.5137 ± 1.503^{fg}
		4	973.5259 ± 0.603^{kl}
		3	862.2227 ± 0.688^{m}
	80	5	1090.9383 ± 6.341^{ef}
		4	768.8918 ± 2.006^{n}
		3	635.9117 ± 3.941^{p}

Different letters denote significant difference ($p<0.05$).

C. Statistical Analyses

Table C. 1. Two-way ANOVA and Tukey's Comparison Test with 95% confidence level for extraction yield of pectin samples extracted by conventional method

General Linear Model: Yield, % versus Temperature; Time

```
Factor       Type   Levels  Values
Temperature  fixed       2  80; 90
Time         fixed       3  3; 4; 5


Analysis of Variance for Yield, %, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS  Adj MS       F      P
Temperature        1   1,6950   1,6950  1,6950   20,03  0,004
Time               2  19,3413  19,3413  9,6707  114,25  0,000
Temperature*Time   2   0,3240   0,3240  0,1620    1,91  0,228
Error              6   0,5078   0,5078  0,0846
Total             11  21,8682


S = 0,290932   R-Sq = 97,68%   R-Sq(adj) = 95,74%


Grouping Information Using Tukey Method and 95,0% Confidence

Temperature  N  Mean  Grouping
90           6   5,4  A
80           6   4,6    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Time  N  Mean  Grouping
5     4   6,1  A
4     4   5,7  A
3     4   3,2    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Temperature  Time  N  Mean  Grouping
90           5     2   6,4  A
90           4     2   6,3  A B
80           5     2   5,7  A B
80           4     2   5,1    B
90           3     2   3,4      C
80           3     2   3,0      C

Means that do not share a letter are significantly different.
```

Table C. 2. Two-way ANOVA and Tukey's Comparison Test with 95% confidence level for degree of esterification of pectin samples extracted by conventional method

General Linear Model: DE Value, % versus Temperature; Time

```
Factor       Type   Levels  Values
Temperature  fixed       2  80; 90
Time         fixed       3  3; 4; 5


Analysis of Variance for DE Value, %, using Adjusted SS for Tests

Source            DF   Seq SS   Adj SS   Adj MS        F      P
Temperature        1  20,1243  20,1243  20,1243  4281,77  0,000
Time               2  21,8097  21,8097  10,9049  2320,18  0,000
Temperature*Time   2   2,4069   2,4069   1,2035   256,06  0,000
Error              6   0,0282   0,0282   0,0047
Total             11  44,3692


S = 0,0685565   R-Sq = 99,94%   R-Sq(adj) = 99,88%


Unusual Observations for DE Value, %

Obs  DE Value, %      Fit  SE Fit  Residual  St Resid
  3      38,0000  37,9000  0,0485    0,1000      2,06 R
  4      37,8000  37,9000  0,0485   -0,1000     -2,06 R

R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95,0% Confidence

Temperature  N  Mean  Grouping
80           6  37,7  A
90           6  35,1    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Time  N  Mean  Grouping
5     4  38,2  A
4     4  36,0    B
3     4  35,0      C

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Temperature  Time  N  Mean  Grouping
80           5     2  39,2  A
80           4     2  37,9    B
90           5     2  37,3      C
80           3     2  36,0        D
90           4     2  34,0          E
```

90	3	2	34,0	E

Means that do not share a letter are significantly different.

Table C. 3. Two-way ANOVA and Tukey's Comparison Test with 95% confidence level for viscosity of pectin samples extracted by conventional method

General Linear Model: Viscosity, Pa.s versus Temperature; Time

```
Factor       Type   Levels  Values
Temperature  fixed       2  80; 90
Time         fixed       3  3; 4; 5


Analysis of Variance for Viscosity, Pa.s, using Adjusted SS for Tests

Source            DF     Seq SS     Adj SS     Adj MS     F      P
Temperature        1  0,0000000  0,0000000  0,0000000  0,22  0,652
Time               2  0,0000000  0,0000000  0,0000000  1,99  0,218
Temperature*Time   2  0,0000000  0,0000000  0,0000000  0,69  0,536
Error              6  0,0000000  0,0000000  0,0000000
Total             11  0,0000001


S = 0,0000852447   R-Sq = 48,20%   R-Sq(adj) = 5,03%


Grouping Information Using Tukey Method and 95,0% Confidence

Temperature  N  Mean  Grouping
80           6   0,0  A
90           6   0,0  A

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Time  N  Mean  Grouping
3     4   0,0  A
4     4   0,0  A
5     4   0,0  A

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Temperature  Time  N  Mean  Grouping
80           3     2   0,0  A
90           3     2   0,0  A
80           4     2   0,0  A
90           4     2   0,0  A
90           5     2   0,0  A
80           5     2   0,0  A

Means that do not share a letter are significantly different.
```

Table C. 4. Two-way ANOVA and Tukey's Comparison Test with 95% confidence level for galacturonic acid content of pectin samples extracted by conventional method

General Linear Model: Gal-A, % versus Temperature; Time

```
Factor       Type   Levels  Values
Temperature  fixed       2  80; 90
Time         fixed       3  3; 4; 5


Analysis of Variance for Gal-A, %, using Adjusted SS for Tests

Source            DF   Seq SS  Adj SS  Adj MS      F      P
Temperature        1   9,8211  9,8211  9,8211  74,97  0,000
Time               2   5,6322  5,6322  2,8161  21,50  0,002
Temperature*Time   2   0,0785  0,0785  0,0392   0,30  0,752
Error              6   0,7859  0,7859  0,1310
Total             11  16,3177


S = 0,361928   R-Sq = 95,18%   R-Sq(adj) = 91,17%


Grouping Information Using Tukey Method and 95,0% Confidence

Temperature  N  Mean  Grouping
80           6  79,8  A
90           6  78,0    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Time  N  Mean  Grouping
3     4  79,7  A
4     4  79,0  A
5     4  78,0    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Temperature  Time  N  Mean  Grouping
80           3     2  80,6  A
80           4     2  79,9  A B
80           5     2  79,0    B C
90           3     2  78,8    B C
90           4     2  78,2      C D
90           5     2  77,0        D

Means that do not share a letter are significantly different.
```

Table C. 5. Two-way ANOVA and Tukey's Comparison Test with 95% confidence level for T2 value of pectin samples extracted by conventional method

General Linear Model: T2, ms versus Temperature; Time

```
Factor       Type   Levels  Values
Temperature  fixed       2  80; 90
Time         fixed       3  3; 4; 5


Analysis of Variance for T2, ms, using Adjusted SS for Tests

Source            DF  Seq SS  Adj SS  Adj MS         F      P
Temperature        1   81713   81713   81713  58427,76  0,000
Time               2  199666  199666   99833  71384,08  0,000
Temperature*Time   2   28453   28453   14227  10172,59  0,000
Error              6       8       8       1
Total             11  309841


S = 1,18260   R-Sq = 100,00%   R-Sq(adj) = 100,00%


Grouping Information Using Tukey Method and 95,0% Confidence

Temperature  N   Mean  Grouping
90           6  965,0  A
80           6  800,0    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Time  N    Mean  Grouping
5     4  1056,9  A
4     4   841,4    B
3     4   749,1      C

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Temperature  Time  N    Mean  Grouping
90           5     2  1072,3  A
80           5     2  1041,6    B
90           4     2   970,6      C
90           3     2   852,1        D
80           4     2   712,3          E
80           3     2   646,0            F

Means that do not share a letter are significantly different.
```

Table C. 6. Three-way ANOVA and Tukey's Comparison Test with 95% confidence level for pectin extraction yield of samples pressurized with acid on HHP assisted and conventional extraction methods

General Linear Model: Yield, % versus Pressure; Temperature; Time

```
Factor       Type   Levels  Values
Pressure     fixed       4  0,1; 250,0; 350,0; 450,0
Temperature  fixed       2  80; 90
Time         fixed       3  3; 4; 5


Analysis of Variance for Yield, %, using Adjusted SS for Tests

Source                     DF   Seq SS   Adj SS  Adj MS        F       P
Pressure                    3  161,558  161,558  53,853  2073,74  0,000
Temperature                 1   20,449   20,449  20,449   787,46  0,000
Time                        2   63,247   63,247  31,624  1217,76  0,000
Pressure*Temperature        3    3,849    3,849   1,283    49,41  0,000
Pressure*Time               6    6,996    6,996   1,166    44,90  0,000
Temperature*Time            2    1,550    1,550   0,775    29,84  0,000
Pressure*Temperature*Time   6    0,376    0,376   0,063     2,41  0,058
Error                      24    0,623    0,623   0,026
Total                      47  258,648


S = 0,161148   R-Sq = 99,76%   R-Sq(adj) = 99,53%


Unusual Observations for Yield, %

Obs  Yield, %     Fit  SE Fit  Residual  St Resid
  3    4,7500  5,1050  0,1139   -0,3550     -3,12 R
  4    5,4600  5,1050  0,1139    0,3550      3,12 R

R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95,0% Confidence

Pressure   N  Mean  Grouping
450,0     12   9,8  A
350,0     12   7,8     B
250,0     12   5,9        C
  0,1     12   5,0           D

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Temperature   N  Mean  Grouping
90           24   7,8  A
80           24   6,5     B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence
```

Time	N	Mean	Grouping
5	16	8,3	A
4	16	7,5	B
3	16	5,6	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	Temperature	N	Mean	Grouping
450,0	90	6	10,8	A
450,0	80	6	8,7	B
350,0	90	6	8,6	B
350,0	80	6	7,1	C
250,0	90	6	6,3	D
250,0	80	6	5,5	E
0,1	90	6	5,4	E
0,1	80	6	4,6	F

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	Time	N	Mean	Grouping
450,0	5	4	11,2	A
450,0	4	4	9,6	B
350,0	5	4	8,5	C
450,0	3	4	8,4	C
350,0	4	4	8,1	C
250,0	5	4	7,5	D
350,0	3	4	6,9	E
250,0	4	4	6,4	F
0,1	5	4	6,1	F G
0,1	4	4	5,7	G
250,0	3	4	3,8	H
0,1	3	4	3,2	I

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Temperature	Time	N	Mean	Grouping
90	5	8	9,0	A
90	4	8	8,3	B
80	5	8	7,6	C
80	4	8	6,6	D
90	3	8	6,0	E
80	3	8	5,2	F

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	Temperature	Time	N	Mean	Grouping
450,0	90	5	2	12,2	A

450,0	90	4	2	10,9	B							
450,0	80	5	2	10,2	B							
350,0	90	5	2	9,5		C						
450,0	90	3	2	9,3		C						
350,0	90	4	2	9,0		C	D					
450,0	80	4	2	8,4			D	E				
250,0	90	5	2	7,9				E	F			
350,0	80	5	2	7,5					F	G		
450,0	80	3	2	7,5					F	G		
350,0	90	3	2	7,4					F	G		
350,0	80	4	2	7,3						G		
250,0	80	5	2	7,1						G		
250,0	90	4	2	7,1						G		
350,0	80	3	2	6,5							H	
0,1	90	5	2	6,4							H	
0,1	90	4	2	6,3							H	I
250,0	80	4	2	5,7								I J
0,1	80	5	2	5,7								I J
0,1	80	4	2	5,1								J
250,0	90	3	2	3,9								K
250,0	80	3	2	3,7								K
0,1	90	3	2	3,4								K L
0,1	80	3	2	3,0								L

Means that do not share a letter are significantly different.

Table C. 7. Three-way ANOVA and Tukey's Comparison Test with 95% confidence level for pectin extraction yield of samples pressurized without acid on HHP assisted and conventional extraction methods

General Linear Model: Yield, % versus Pressure; Temperature; Time

```
Factor       Type   Levels  Values
Pressure     fixed       4  0,1; 250,0; 350,0; 450,0
Temperature  fixed       2  80; 90
Time         fixed       3  3; 4; 5


Analysis of Variance for Yield, %, using Adjusted SS for Tests

Source                      DF    Seq SS    Adj SS   Adj MS        F      P
Pressure                     3  125,2153  125,2153  41,7384  1626,57  0,000
Temperature                  1   23,1991   23,1991  23,1991   904,08  0,000
Time                         2   48,0391   48,0391  24,0196   936,05  0,000
Pressure*Temperature         3    3,7155    3,7155   1,2385    48,26  0,000
Pressure*Time                6    7,9016    7,9016   1,3169    51,32  0,000
Temperature*Time             2    2,5233    2,5233   1,2616    49,17  0,000
Pressure*Temperature*Time    6    1,9719    1,9719   0,3286    12,81  0,000
Error                       24    0,6159    0,6159   0,0257
Total                       47  213,1816


S = 0,160189   R-Sq = 99,71%   R-Sq(adj) = 99,43%


Unusual Observations for Yield, %

Obs  Yield, %     Fit  SE Fit  Residual  St Resid
  3    4,7500  5,1050  0,1133   -0,3550     -3,13 R
  4    5,4600  5,1050  0,1133    0,3550      3,13 R

R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95,0% Confidence

Pressure   N  Mean  Grouping
450,0     12   9,0  A
350,0     12   7,5    B
250,0     12   5,4      C
  0,1     12   5,0        D

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Temperature   N  Mean  Grouping
90           24   7,4  A
80           24   6,0    B

Means that do not share a letter are significantly different.
```

Grouping Information Using Tukey Method and 95,0% Confidence

Time	N	Mean	Grouping
5	16	7,8	A
4	16	7,0	B
3	16	5,4	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	Temperature	N	Mean	Grouping
450,0	90	6	10,1	A
350,0	90	6	8,2	B
450,0	80	6	7,8	C
350,0	80	6	6,8	D
250,0	90	6	6,0	E
0,1	90	6	5,4	F
250,0	80	6	4,9	G
0,1	80	6	4,6	G

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	Time	N	Mean	Grouping
450,0	5	4	10,3	A
450,0	4	4	8,7	B
350,0	5	4	8,1	C
450,0	3	4	7,9	C D
350,0	4	4	7,6	D
350,0	3	4	6,9	E
250,0	5	4	6,7	E
0,1	5	4	6,1	F
250,0	4	4	6,0	F
0,1	4	4	5,7	F
250,0	3	4	3,6	G
0,1	3	4	3,2	G

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Temperature	Time	N	Mean	Grouping
90	5	8	8,8	A
90	4	8	7,7	B
80	5	8	6,8	C
80	4	8	6,3	D
90	3	8	5,8	E
80	3	8	5,0	F

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	Temperature	Time	N	Mean	Grouping

450,0	90	5	2	12,1	A
450,0	90	4	2	9,5	B
350,0	90	5	2	9,0	B C
450,0	90	3	2	8,8	C
450,0	80	5	2	8,5	C D
350,0	90	4	2	8,3	C D
450,0	80	4	2	8,0	D E
250,0	90	5	2	7,5	E F
350,0	90	3	2	7,3	E F G
350,0	80	5	2	7,2	F G
450,0	80	3	2	7,0	F G H
350,0	80	4	2	6,9	F G H I
250,0	90	4	2	6,8	G H I
0,1	90	5	2	6,4	H I J
350,0	80	3	2	6,4	H I J
0,1	90	4	2	6,3	I J K
250,0	80	5	2	5,9	J K
0,1	80	5	2	5,7	K L
250,0	80	4	2	5,2	L
0,1	80	4	2	5,1	L
250,0	90	3	2	3,7	M
250,0	80	3	2	3,5	M N
0,1	90	3	2	3,4	M N
0,1	80	3	2	3,0	N

Means that do not share a letter are significantly different.

Table C. 8. Three-way ANOVA and Tukey's Comparison Test with 95% confidence level for degree of esterification of samples pressurized with acid on HHP assisted and conventional extraction methods

General Linear Model: DE, % versus Pressure; Temperature; Time

```
Factor       Type   Levels  Values
Pressure     fixed       4  0,1; 250,0; 350,0; 450,0
Temperature  fixed       2  80; 90
Time         fixed       3  3; 4; 5


Analysis of Variance for DE, %, using Adjusted SS for Tests

Source                     DF    Seq SS   Adj SS   Adj MS       F      P
Pressure                    3   25,6027  25,6027   8,5342   35,03  0,000
Temperature                 1   69,6490  69,6490  69,6490  285,89  0,000
Time                        2   43,8789  43,8789  21,9395   90,05  0,000
Pressure*Temperature        3    6,7415   6,7415   2,2472    9,22  0,000
Pressure*Time               6    9,2885   9,2885   1,5481    6,35  0,000
Temperature*Time            2    1,6217   1,6217   0,8108    3,33  0,053
Pressure*Temperature*Time   6    7,3937   7,3937   1,2323    5,06  0,002
Error                      24    5,8470   5,8470   0,2436
Total                      47  170,0230


S = 0,493584   R-Sq = 96,56%   R-Sq(adj) = 93,27%


Unusual Observations for DE, %

Obs    DE, %      Fit  SE Fit  Residual  St Resid
 17  39,0000  38,0000  0,3490    1,0000      2,87 R
 18  37,0000  38,0000  0,3490   -1,0000     -2,87 R
 19  34,0000  33,0000  0,3490    1,0000      2,87 R
 20  32,0000  33,0000  0,3490   -1,0000     -2,87 R

R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95,0% Confidence

Pressure   N  Mean  Grouping
250,0     12  36,6  A
  0,1     12  36,4  A
350,0     12  35,6     B
450,0     12  34,8       C

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Temperature   N  Mean  Grouping
80           24  37,1  A
90           24  34,6     B

Means that do not share a letter are significantly different.
```

Grouping Information Using Tukey Method and 95,0% Confidence

Time	N	Mean	Grouping
5	16	37,0	A
4	16	35,9	B
3	16	34,7	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	Temperature	N	Mean	Grouping
0,1	80	6	37,7	A
250,0	80	6	37,2	A B
350,0	80	6	37,0	A B C
450,0	80	6	36,3	B C
250,0	90	6	36,1	C
0,1	90	6	35,1	D
350,0	90	6	34,1	E
450,0	90	6	33,2	E

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	Time	N	Mean	Grouping
0,1	5	4	38,2	A
250,0	4	4	37,6	A B
250,0	5	4	37,3	A B
350,0	5	4	36,7	B C
0,1	4	4	36,0	C D
450,0	5	4	35,8	C D E
350,0	4	4	35,3	D E
0,1	3	4	35,0	D E F
250,0	3	4	35,0	D E F
350,0	3	4	34,7	E F
450,0	4	4	34,6	E F
450,0	3	4	34,0	F

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Temperature	Time	N	Mean	Grouping
80	5	8	38,3	A
80	4	8	37,3	B
90	5	8	35,8	C
80	3	8	35,6	C
90	4	8	34,5	D
90	3	8	33,7	D

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	Temperature	Time	N	Mean	Grouping
0,1	80	5	2	39,2	A
350,0	80	5	2	38,4	A B
250,0	80	4	2	38,1	A B
450,0	80	5	2	38,0	A B C
0,1	80	4	2	37,9	A B C
250,0	80	5	2	37,5	A B C D
0,1	90	5	2	37,3	A B C D
250,0	90	5	2	37,2	A B C D
350,0	80	4	2	37,0	B C D E
250,0	90	4	2	37,0	B C D E
0,1	80	3	2	36,0	C D E F
450,0	80	4	2	36,0	C D E F G
250,0	80	3	2	36,0	C D E F G
350,0	80	3	2	35,5	D E F G H
450,0	80	3	2	35,0	E F G H I
350,0	90	5	2	35,0	E F G H I
0,1	90	4	2	34,0	F G H I
250,0	90	3	2	34,0	G H I
0,1	90	3	2	34,0	G H I
350,0	90	3	2	33,9	H I
350,0	90	4	2	33,6	H I
450,0	90	5	2	33,5	H I
450,0	90	4	2	33,2	I
450,0	90	3	2	33,0	I

Means that do not share a letter are significantly different.

Table C. 9. Three-way ANOVA and Tukey's Comparison Test with 95% confidence level for degree of esterification of samples pressurized without acid on HHP assisted and conventional extraction methods

General Linear Model: DE, % versus Pressure; Temperature; Time

```
Factor       Type   Levels  Values
Pressure     fixed       4  0,1; 250,0; 350,0; 450,0
Temperature  fixed       2  80; 90
Time         fixed       3  3; 4; 5


Analysis of Variance for DE, %, using Adjusted SS for Tests

Source                      DF    Seq SS   Adj SS   Adj MS       F      P
Pressure                     3   61,2663  61,2663  20,4221  454,33  0,000
Temperature                  1   21,9511  21,9511  21,9511  488,34  0,000
Time                         2   31,7738  31,7738  15,8869  353,43  0,000
Pressure*Temperature         3    7,8509   7,8509   2,6170   58,22  0,000
Pressure*Time                6    8,2969   8,2969   1,3828   30,76  0,000
Temperature*Time             2    4,0974   4,0974   2,0487   45,58  0,000
Pressure*Temperature*Time    6    6,2845   6,2845   1,0474   23,30  0,000
Error                       24    1,0788   1,0788   0,0450
Total                       47  142,5996


S = 0,212014   R-Sq = 99,24%   R-Sq(adj) = 98,52%


Unusual Observations for DE, %

Obs    DE, %      Fit  SE Fit  Residual  St Resid
 35  35,1900  34,6900  0,1499    0,5000      3,34 R
 36  34,1900  34,6900  0,1499   -0,5000     -3,34 R

R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95,0% Confidence

Pressure   N  Mean  Grouping
  0,1     12  36,4  A
250,0     12  34,0    B
350,0     12  33,9    B
450,0     12  33,6      C

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Temperature   N  Mean  Grouping
80           24  35,1  A
90           24  33,8    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence
```

Time	N	Mean	Grouping
5	16	35,6	A
4	16	34,3	B
3	16	33,6	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	Temperature	N	Mean	Grouping
0,1	80	6	37,7	A
0,1	90	6	35,1	B
450,0	80	6	34,3	C
350,0	80	6	34,3	C
250,0	80	6	34,3	C
250,0	90	6	33,8	D
350,0	90	6	33,5	D
450,0	90	6	32,8	E

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	Time	N	Mean	Grouping
0,1	5	4	38,2	A
0,1	4	4	36,0	B
250,0	5	4	35,4	C
0,1	3	4	35,0	C D
350,0	5	4	34,7	D
350,0	4	4	33,9	E
450,0	5	4	33,9	E
450,0	4	4	33,6	E F
250,0	4	4	33,6	E F G
450,0	3	4	33,2	F G
350,0	3	4	33,1	F G
250,0	3	4	33,1	G

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Temperature	Time	N	Mean	Grouping
80	5	8	35,8	A
90	5	8	35,3	B
80	4	8	35,1	B
80	3	8	34,5	C
90	4	8	33,4	D
90	3	8	32,7	E

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	Temperature	Time	N	Mean	Grouping
0,1	80	5	2	39,2	A

0,1	80	4	2	37,9	B				
0,1	90	5	2	37,3	B				
250,0	90	5	2	36,2		C			
0,1	80	3	2	36,0		C			
450,0	80	5	2	34,7			D		
350,0	90	5	2	34,7			D		
350,0	80	5	2	34,7			D		
250,0	80	5	2	34,7			D		
450,0	80	4	2	34,3			D	E	
350,0	80	4	2	34,2			D	E	
250,0	80	4	2	34,1			D	E	
0,1	90	4	2	34,0			D	E	
0,1	90	3	2	34,0			D	E	
350,0	80	3	2	34,0			D	E	
250,0	80	3	2	34,0			D	E	
450,0	80	3	2	34,0			D	E	
350,0	90	4	2	33,6				E	F
450,0	90	5	2	33,0				F	G
450,0	90	4	2	33,0				F	G
250,0	90	4	2	33,0				F	G
450,0	90	3	2	32,4					G
350,0	90	3	2	32,2					G
250,0	90	3	2	32,1					G

Means that do not share a letter are significantly different.

Table C. 10. Three-way ANOVA and Tukey's Comparison Test with 95% confidence level for viscosity of samples pressurized with acid on HHP assisted and conventional extraction methods

General Linear Model: Viscosity, (Pa.s) versus Pressure; Temperature; Time

```
Factor       Type   Levels  Values
Pressure     fixed       4  0,1; 250,0; 350,0; 450,0
Temperature  fixed       2  80; 90
Time         fixed       3  3; 4; 5


Analysis of Variance for Viscosity (Pa.s), using Adjusted SS for Tests

Source                     DF     Seq SS     Adj SS     Adj MS     F      P
Pressure                    3  0,0000001  0,0000001  0,0000000  5,01  0,008
Temperature                 1  0,0000000  0,0000000  0,0000000  6,02  0,022
Time                        2  0,0000000  0,0000000  0,0000000  0,81  0,456
Pressure*Temperature        3  0,0000000  0,0000000  0,0000000  0,43  0,731
Pressure*Time               6  0,0000001  0,0000001  0,0000000  3,02  0,024
Temperature*Time            2  0,0000000  0,0000000  0,0000000  0,06  0,940
Pressure*Temperature*Time   6  0,0000000  0,0000000  0,0000000  0,94  0,484
Error                      24  0,0000001  0,0000001  0,0000000
Total                      47  0,0000003


S = 0,0000611692   R-Sq = 66,60%   R-Sq(adj) = 34,59%


Unusual Observations for Viscosity (Pa.s)

     Viscosity
Obs     (Pa.s)       Fit    SE Fit   Residual  St Resid
  3   0,003810  0,003930  0,000043  -0,000120     -2,77 R
  4   0,004050  0,003930  0,000043   0,000120      2,77 R
 41   0,003990  0,004120  0,000043  -0,000130     -3,01 R
 42   0,004250  0,004120  0,000043   0,000130      3,01 R

R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95,0% Confidence

Pressure   N  Mean  Grouping
250,0     12   0,0  A
350,0     12   0,0  A B
  0,1     12   0,0    B
450,0     12   0,0    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Temperature   N  Mean  Grouping
80           24   0,0  A
90           24   0,0    B

Means that do not share a letter are significantly different.
```

Grouping Information Using Tukey Method and 95,0% Confidence

Time	N	Mean	Grouping
5	16	0,0	A
4	16	0,0	A
3	16	0,0	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	Temperature	N	Mean	Grouping
250,0	80	6	0,0	A
250,0	90	6	0,0	A B
350,0	80	6	0,0	A B
450,0	80	6	0,0	A B
0,1	80	6	0,0	A B
350,0	90	6	0,0	A B
0,1	90	6	0,0	A B
450,0	90	6	0,0	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	Time	N	Mean	Grouping
250,0	5	4	0,0	A
0,1	3	4	0,0	A B
350,0	5	4	0,0	A B
250,0	4	4	0,0	A B
250,0	3	4	0,0	A B
350,0	4	4	0,0	A B
450,0	5	4	0,0	A B
450,0	4	4	0,0	A B
0,1	4	4	0,0	A B
350,0	3	4	0,0	B
450,0	3	4	0,0	B
0,1	5	4	0,0	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Temperature	Time	N	Mean	Grouping
80	5	8	0,0	A
80	4	8	0,0	A
80	3	8	0,0	A
90	5	8	0,0	A
90	4	8	0,0	A
90	3	8	0,0	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	Temperature	Time	N	Mean	Grouping
250,0	80	5	2	0,0	A
0,1	80	3	2	0,0	A B
250,0	80	4	2	0,0	A B
350,0	90	5	2	0,0	A B
350,0	80	5	2	0,0	A B
250,0	80	3	2	0,0	A B
250,0	90	5	2	0,0	A B
250,0	90	4	2	0,0	A B
250,0	90	3	2	0,0	A B
450,0	80	5	2	0,0	A B
350,0	80	4	2	0,0	A B
450,0	80	4	2	0,0	A B
350,0	90	4	2	0,0	A B
0,1	90	3	2	0,0	A B
0,1	80	4	2	0,0	A B
0,1	90	4	2	0,0	A B
450,0	80	3	2	0,0	A B
350,0	80	3	2	0,0	A B
0,1	90	5	2	0,0	A B
450,0	90	5	2	0,0	A B
450,0	90	4	2	0,0	A B
350,0	90	3	2	0,0	B
450,0	90	3	2	0,0	B
0,1	80	5	2	0,0	B

Means that do not share a letter are significantly different.

Table C. 11. Three-way ANOVA and Tukey's Comparison Test with 95% confidence level for viscosity of samples pressurized without acid on HHP assisted and conventional extraction methods

General Linear Model: Viscosity, (Pa.s) versus Pressure; Temperature; Time

```
Factor       Type   Levels  Values
Pressure     fixed       4  0,1; 250,0; 350,0; 450,0
Temperature  fixed       2  80; 90
Time         fixed       3  3; 4; 5


Analysis of Variance for Viscosity (Pa.s), using Adjusted SS for Tests

Source                     DF     Seq SS     Adj SS     Adj MS      F      P
Pressure                    3  0,0000000  0,0000000  0,0000000   2,53  0,081
Temperature                 1  0,0000001  0,0000001  0,0000001  24,26  0,000
Time                        2  0,0000000  0,0000000  0,0000000   1,95  0,165
Pressure*Temperature        3  0,0000000  0,0000000  0,0000000   1,87  0,162
Pressure*Time               6  0,0000000  0,0000000  0,0000000   1,03  0,429
Temperature*Time            2  0,0000000  0,0000000  0,0000000   1,72  0,201
Pressure*Temperature*Time   6  0,0000000  0,0000000  0,0000000   0,50  0,804
Error                      24  0,0000001  0,0000001  0,0000000
Total                      47  0,0000003


S = 0,0000627163   R-Sq = 69,21%   R-Sq(adj) = 39,70%


Unusual Observations for Viscosity (Pa.s)

     Viscosity
Obs     (Pa.s)       Fit    SE Fit   Residual  St Resid
  3   0,003810  0,003930  0,000044  -0,000120     -2,71 R
  4   0,004050  0,003930  0,000044   0,000120      2,71 R

R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95,0% Confidence

Pressure   N  Mean  Grouping
250,0     12   0,0  A
350,0     12   0,0  A
  0,1     12   0,0  A
450,0     12   0,0  A

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Temperature   N  Mean  Grouping
80           24   0,0  A
90           24   0,0    B

Means that do not share a letter are significantly different.
```

Grouping Information Using Tukey Method and 95,0% Confidence

Time	N	Mean	Grouping
3	16	0,0	A
4	16	0,0	A
5	16	0,0	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	Temperature	N	Mean	Grouping
250,0	80	6	0,0	A
350,0	80	6	0,0	A
450,0	80	6	0,0	A
0,1	80	6	0,0	A
0,1	90	6	0,0	A B
250,0	90	6	0,0	A B
350,0	90	6	0,0	A B
450,0	90	6	0,0	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	Time	N	Mean	Grouping
0,1	3	4	0,0	A
250,0	3	4	0,0	A
350,0	5	4	0,0	A
250,0	4	4	0,0	A
250,0	5	4	0,0	A
350,0	3	4	0,0	A
0,1	4	4	0,0	A
350,0	4	4	0,0	A
450,0	4	4	0,0	A
450,0	3	4	0,0	A
0,1	5	4	0,0	A
450,0	5	4	0,0	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Temperature	Time	N	Mean	Grouping
80	3	8	0,0	A
80	4	8	0,0	A B
80	5	8	0,0	A B C
90	3	8	0,0	B C
90	5	8	0,0	B C
90	4	8	0,0	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	Temperature	Time	N	Mean	Grouping
0,1	80	3	2	0,0	A
250,0	80	4	2	0,0	A
250,0	80	3	2	0,0	A
350,0	80	3	2	0,0	A
250,0	80	5	2	0,0	A
350,0	80	4	2	0,0	A
450,0	80	4	2	0,0	A
450,0	80	3	2	0,0	A
350,0	80	5	2	0,0	A
350,0	90	5	2	0,0	A
0,1	90	3	2	0,0	A
0,1	80	4	2	0,0	A
250,0	90	3	2	0,0	A
0,1	90	4	2	0,0	A
450,0	80	5	2	0,0	A
250,0	90	4	2	0,0	A
0,1	90	5	2	0,0	A
250,0	90	5	2	0,0	A
350,0	90	3	2	0,0	A
0,1	80	5	2	0,0	A
350,0	90	4	2	0,0	A
450,0	90	4	2	0,0	A
450,0	90	3	2	0,0	A
450,0	90	5	2	0,0	A

Means that do not share a letter are significantly different.

Table C. 12. Three-way ANOVA and Tukey's Comparison Test with 95% confidence level for galacturonic acid content of samples pressurized with acid on HHP assisted and conventional extraction methods

General Linear Model: Gal-A, % versus Pressure; Temperature; Time

```
Factor       Type   Levels  Values
Pressure     fixed       4  0,1; 250,0; 350,0; 450,0
Temperature  fixed       2  80; 90
Time         fixed       3  3; 4; 5


Analysis of Variance for Gal-A, %, using Adjusted SS for Tests

Source                     DF   Seq SS   Adj SS   Adj MS        F      P
Pressure                    3  665,488  665,488  221,829  1423,75  0,000
Temperature                 1   80,471   80,471   80,471   516,48  0,000
Time                        2   48,576   48,576   24,288   155,89  0,000
Pressure*Temperature        3   16,870   16,870    5,623    36,09  0,000
Pressure*Time               6    5,300    5,300    0,883     5,67  0,001
Temperature*Time            2    3,691    3,691    1,846    11,84  0,000
Pressure*Temperature*Time   6    2,803    2,803    0,467     3,00  0,025
Error                      24    3,739    3,739    0,156
Total                      47  826,938


S = 0,394723   R-Sq = 99,55%   R-Sq(adj) = 99,11%


Grouping Information Using Tukey Method and 95,0% Confidence

Pressure   N  Mean  Grouping
  0,1     12  78,9  A
250,0     12  74,2    B
350,0     12  71,8      C
450,0     12  68,7        D

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Temperature   N  Mean  Grouping
80           24  74,7  A
90           24  72,1    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Time   N  Mean  Grouping
3     16  74,5  A
4     16  73,6    B
5     16  72,1      C

Means that do not share a letter are significantly different.
```

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	Temperature	N	Mean	Grouping
0,1	80	6	79,8	A
0,1	90	6	78,0	B
250,0	80	6	75,2	C
250,0	90	6	73,1	D
350,0	80	6	72,8	D
450,0	80	6	71,0	E
350,0	90	6	70,9	E
450,0	90	6	66,4	F

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	Time	N	Mean	Grouping
0,1	3	4	79,7	A
0,1	4	4	79,0	A
0,1	5	4	78,0	B
250,0	3	4	75,0	C
250,0	4	4	74,4	C
250,0	5	4	73,0	D
350,0	3	4	73,0	D
350,0	4	4	71,8	E
350,0	5	4	70,7	F
450,0	3	4	70,4	F
450,0	4	4	69,0	G
450,0	5	4	66,7	H

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Temperature	Time	N	Mean	Grouping
80	3	8	75,6	A
80	4	8	74,7	B
80	5	8	73,8	C
90	3	8	73,5	C
90	4	8	72,4	D
90	5	8	70,4	E

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	Temperature	Time	N	Mean	Grouping
0,1	80	3	2	80,6	A
0,1	80	4	2	79,9	A B
0,1	80	5	2	79,0	A B C
0,1	90	3	2	78,8	B C
0,1	90	4	2	78,2	C D
0,1	90	5	2	77,0	D E
250,0	80	3	2	75,9	E F
250,0	80	4	2	75,1	F G
250,0	80	5	2	74,6	F G
250,0	90	3	2	74,2	G H

350,0	80	3	2	73,9	G H
250,0	90	4	2	73,7	G H I
350,0	80	4	2	72,8	H I J
350,0	90	3	2	72,2	I J K
450,0	80	3	2	72,1	J K
350,0	80	5	2	71,7	J K
250,0	90	5	2	71,5	J K
450,0	80	4	2	71,1	K L
350,0	90	4	2	70,9	K L
450,0	80	5	2	69,8	L M
350,0	90	5	2	69,7	L M
450,0	90	3	2	68,8	M
450,0	90	4	2	66,8	N
450,0	90	5	2	63,5	O

Means that do not share a letter are significantly different.

Table C. 13. Three-way ANOVA and Tukey's Comparison Test with 95% confidence level for galacturonic acid content of samples pressurized without acid on HHP assisted and conventional extraction methods

General Linear Model: Gal-A, % versus Pressure; Temperature; Time

Factor	Type	Levels	Values
Pressure	fixed	4	0,1; 250,0; 350,0; 450,0
Temperature	fixed	2	80; 90
Time	fixed	3	3; 4; 5

Analysis of Variance for Gal-A, %, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Pressure	3	371,305	371,305	123,768	840,59	0,000
Temperature	1	127,976	127,976	127,976	869,17	0,000
Time	2	55,387	55,387	27,694	188,09	0,000
Pressure*Temperature	3	44,281	44,281	14,760	100,25	0,000
Pressure*Time	6	4,035	4,035	0,673	4,57	0,003
Temperature*Time	2	2,552	2,552	1,276	8,67	0,001
Pressure*Temperature*Time	6	2,543	2,543	0,424	2,88	0,029
Error	24	3,534	3,534	0,147		
Total	47	611,613				

S = 0,383718 R-Sq = 99,42% R-Sq(adj) = 98,87%

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	N	Mean	Grouping
0,1	12	78,9	A
250,0	12	76,9	B
350,0	12	74,8	C
450,0	12	71,4	D

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Temperature	N	Mean	Grouping
80	24	77,1	A
90	24	73,9	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Time	N	Mean	Grouping
3	16	76,7	A
4	16	75,6	B
5	16	74,1	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	Temperature	N	Mean	Grouping
0,1	80	6	79,8	A
0,1	90	6	78,0	B
250,0	80	6	77,8	B
350,0	80	6	76,3	C
250,0	90	6	76,0	C
450,0	80	6	74,6	D
350,0	90	6	73,3	E
450,0	90	6	68,1	F

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	Time	N	Mean	Grouping
0,1	3	4	79,7	A
0,1	4	4	79,0	A
250,0	3	4	78,0	B
0,1	5	4	78,0	B
250,0	4	4	77,0	C
350,0	3	4	76,4	C D
250,0	5	4	75,7	D E
350,0	4	4	75,2	E
350,0	5	4	72,9	F
450,0	3	4	72,9	F
450,0	4	4	71,3	G
450,0	5	4	69,9	H

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Temperature	Time	N	Mean	Grouping
80	3	8	78,1	A
80	4	8	77,2	B
80	5	8	76,1	C
90	3	8	75,4	D
90	4	8	74,1	E
90	5	8	72,2	F

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	Temperature	Time	N	Mean	Grouping
0,1	80	3	2	80,6	A
0,1	80	4	2	79,9	A B
0,1	80	5	2	79,0	A B C
250,0	80	3	2	78,9	B C D
0,1	90	3	2	78,8	B C D E
0,1	90	4	2	78,2	C D E F
250,0	80	4	2	77,9	C D E F G
350,0	80	3	2	77,4	D E F G H
250,0	90	3	2	77,2	E F G H I
0,1	90	5	2	77,0	F G H I

250,0	80	5	2	76,6	G H I J
350,0	80	4	2	76,4	G H I J
250,0	90	4	2	76,1	H I J K
450,0	80	3	2	75,8	I J K
350,0	90	3	2	75,4	J K L
350,0	80	5	2	75,3	J K L
250,0	90	5	2	74,7	K L M
450,0	80	4	2	74,7	K L M
350,0	90	4	2	73,9	L M
450,0	80	5	2	73,4	M
350,0	90	5	2	70,6	N
450,0	90	3	2	70,0	N
450,0	90	4	2	68,0	O
450,0	90	5	2	66,4	P

Means that do not share a letter are significantly different.

Table C. 14. Three-way ANOVA and Tukey's Comparison Test with 95% confidence level for T2 of samples pressurized with acid on HHP assisted and conventional extraction methods

General Linear Model: T2 versus Pressure; Temperature; Time

```
Factor       Type   Levels  Values
Pressure     fixed       4  0,1; 250,0; 350,0; 450,0
Temperature  fixed       2  80; 90
Time         fixed       3  3; 4; 5


Analysis of Variance for T2, using Adjusted SS for Tests

Source                     DF   Seq SS  Adj SS  Adj MS        F      P
Pressure                    3   773685  773685  257895  5173,55  0,000
Temperature                 1    92551   92551   92551  1856,64  0,000
Time                        2   685382  685382  342691  6874,61  0,000
Pressure*Temperature        3    76982   76982   25661   514,77  0,000
Pressure*Time               6    57090   57090    9515   190,88  0,000
Temperature*Time            2    12639   12639    6319   126,77  0,000
Pressure*Temperature*Time   6    30635   30635    5106   102,43  0,000
Error                      24     1196    1196      50
Total                      47  1730161


S = 7,06037   R-Sq = 99,93%   R-Sq(adj) = 99,86%


Unusual Observations for T2

Obs       T2      Fit  SE Fit  Residual  St Resid
 29  1267,61  1278,92    4,99    -11,32     -2,27 R
 30  1290,24  1278,92    4,99     11,32      2,27 R
 45   993,17  1003,38    4,99    -10,20     -2,04 R
 46  1013,58  1003,38    4,99     10,20      2,04 R

R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95,0% Confidence

Pressure   N    Mean  Grouping
450,0     12  1189,3  A
350,0     12  1148,2    B
250,0     12   963,8      C
  0,1     12   882,5        D

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Temperature   N    Mean  Grouping
90           24  1089,9  A
80           24  1002,1    B

Means that do not share a letter are significantly different.
```

Grouping Information Using Tukey Method and 95,0% Confidence

Time	N	Mean	Grouping
5	16	1203,2	A
4	16	1020,9	B
3	16	913,8	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	Temperature	N	Mean	Grouping
450,0	90	6	1205,6	A
450,0	80	6	1172,9	B
350,0	80	6	1155,2	C
350,0	90	6	1141,3	D
250,0	90	6	1047,5	E
0,1	90	6	965,0	F
250,0	80	6	880,2	G
0,1	80	6	800,0	H

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	Time	N	Mean	Grouping
450,0	5	4	1293,7	A
350,0	5	4	1281,6	A
250,0	5	4	1180,6	B
450,0	4	4	1174,8	B
350,0	4	4	1122,6	C
450,0	3	4	1099,4	D
0,1	5	4	1056,9	E
350,0	3	4	1040,5	E
250,0	4	4	944,7	F
0,1	4	4	841,4	G
250,0	3	4	766,2	H
0,1	3	4	749,1	H

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Temperature	Time	N	Mean	Grouping
90	5	8	1230,2	A
80	5	8	1176,2	B
90	4	8	1059,8	C
80	4	8	981,9	D
90	3	8	979,6	D
80	3	8	848,0	E

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	Temperature	Time	N	Mean	Grouping

450,0	90	5	2	1307,0	A
350,0	90	5	2	1284,3	A B
450,0	80	5	2	1280,5	A B
350,0	80	5	2	1278,9	A B
250,0	90	5	2	1257,3	B
450,0	90	4	2	1183,4	C
450,0	80	4	2	1166,1	C
350,0	80	4	2	1163,2	C
450,0	90	3	2	1126,5	D
250,0	80	5	2	1103,9	D E
350,0	90	4	2	1081,9	E F
0,1	90	5	2	1072,3	F
450,0	80	3	2	1072,2	F
350,0	90	3	2	1057,8	F G
0,1	80	5	2	1041,6	G H
350,0	80	3	2	1023,3	H I
250,0	90	4	2	1003,4	I
0,1	90	4	2	970,6	J
250,0	80	4	2	886,1	K
250,0	90	3	2	881,9	K
0,1	90	3	2	852,1	L
0,1	80	4	2	712,3	M
250,0	80	3	2	650,5	N
0,1	80	3	2	646,0	N

Means that do not share a letter are significantly different.

Table C. 15. Three-way ANOVA and Tukey's Comparison Test with 95% confidence level for T2 of samples pressurized without acid on HHP assisted and conventional extraction methods

General Linear Model: T2 versus Pressure; Temperature; Time

```
Factor       Type   Levels  Values
Pressure     fixed       4  0,1; 250,0; 350,0; 450,0
Temperature  fixed       2  80; 90
Time         fixed       3  3; 4; 5


Analysis of Variance for T2, using Adjusted SS for Tests

Source                     DF   Seq SS  Adj SS  Adj MS         F      P
Pressure                    3   587884  587884  195961   9714,18  0,000
Temperature                 1   154301  154301  154301   7649,01  0,000
Time                        2   581230  581230  290615  14406,34  0,000
Pressure*Temperature        3    22431   22431    7477    370,64  0,000
Pressure*Time               6    47544   47544    7924    392,80  0,000
Temperature*Time            2    75782   75782   37891   1878,33  0,000
Pressure*Temperature*Time   6    52020   52020    8670    429,79  0,000
Error                      24      484     484      20
Total                      47  1521676


S = 4,49140   R-Sq = 99,97%   R-Sq(adj) = 99,94%


Unusual Observations for T2

Obs       T2      Fit  SE Fit  Residual  St Resid
 23  1298,28  1305,31    3,18     -7,04     -2,22 R
 24  1312,35  1305,31    3,18      7,04      2,22 R

R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95,0% Confidence

Pressure   N    Mean  Grouping
450,0     12  1162,6  A
350,0     12  1000,8    B
250,0     12   903,2      C
  0,1     12   882,5        D

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Temperature   N    Mean  Grouping
90           24  1044,0  A
80           24   930,6    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence
```

Time	N	Mean	Grouping
5	16	1131,7	A
4	16	965,1	B
3	16	864,9	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	Temperature	N	Mean	Grouping
450,0	90	6	1189,8	A
450,0	80	6	1135,5	B
350,0	90	6	1046,7	C
250,0	90	6	974,4	D
0,1	90	6	965,0	E
350,0	80	6	954,9	F
250,0	80	6	831,9	G
0,1	80	6	800,0	H

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	Time	N	Mean	Grouping
450,0	5	4	1270,7	A
450,0	4	4	1124,4	B
350,0	5	4	1110,1	C
450,0	3	4	1092,8	D
250,0	5	4	1089,2	D
0,1	5	4	1056,9	E
350,0	4	4	1023,4	F
250,0	4	4	871,2	G
350,0	3	4	868,8	G
0,1	4	4	841,4	H
250,0	3	4	749,1	I
0,1	3	4	749,1	I

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Temperature	Time	N	Mean	Grouping
90	5	8	1134,4	A
80	5	8	1129,1	A
90	4	8	1035,4	B
90	3	8	962,1	C
80	4	8	894,8	D
80	3	8	767,8	E

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	Temperature	Time	N	Mean	Grouping
450,0	90	5	2	1305,3	A
450,0	80	5	2	1236,1	B

350,0	80	5	2	1147,6	C
450,0	90	4	2	1142,3	C
450,0	90	3	2	1121,8	D
450,0	80	4	2	1106,6	D E
250,0	80	5	2	1090,9	E F
250,0	90	5	2	1087,5	F G
350,0	90	5	2	1072,6	G H
0,1	90	5	2	1072,3	G H
450,0	80	3	2	1063,8	H
350,0	90	4	2	1055,2	H I
0,1	80	5	2	1041,6	I
350,0	90	3	2	1012,2	J
350,0	80	4	2	991,6	K
250,0	90	4	2	973,5	K L
0,1	90	4	2	970,6	L
250,0	90	3	2	862,2	M
0,1	90	3	2	852,1	M
250,0	80	4	2	768,9	N
350,0	80	3	2	725,5	O
0,1	80	4	2	712,3	O
0,1	80	3	2	646,0	P
250,0	80	3	2	635,9	P

Means that do not share a letter are significantly different.

Table C. 16. Three-way ANOVA and Tukey's Comparison Test with 95% confidence level for T2 of samples pressurized with acid on HHP assisted and conventional extraction methods

General Linear Model: Yield, % versus Pressure; Temperature; Time

```
Factor       Type   Levels  Values
Pressure     fixed       3  250; 350; 450
Temperature  fixed       2  80; 90
Time         fixed       3  3; 4; 5


Analysis of Variance for Yield, %, using Adjusted SS for Tests

Source                     DF    Seq SS   Adj SS   Adj MS        F      P
Pressure                    2   87,7838  87,7838  43,8919  6846,22  0,000
Temperature                 1   19,9809  19,9809  19,9809  3116,60  0,000
Time                        2   44,9580  44,9580  22,4790  3506,26  0,000
Pressure*Temperature        2    2,6225   2,6225   1,3112   204,53  0,000
Pressure*Time               4    5,9435   5,9435   1,4859   231,77  0,000
Temperature*Time            2    1,2426   1,2426   0,6213    96,91  0,000
Pressure*Temperature*Time   4    0,3589   0,3589   0,0897    14,00  0,000
Error                      18    0,1154   0,1154   0,0064
Total                      35  163,0056


S = 0,0800694   R-Sq = 99,93%   R-Sq(adj) = 99,86%


Unusual Observations for Yield, %

Obs  Yield, %      Fit  SE Fit  Residual  St Resid
  5   10,1100  10,2300  0,0566   -0,1200     -2,12 R
  6   10,3500  10,2300  0,0566    0,1200      2,12 R
 11   12,1000  12,2300  0,0566   -0,1300     -2,30 R
 12   12,3600  12,2300  0,0566    0,1300      2,30 R

R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95,0% Confidence

Pressure   N  Mean  Grouping
450       12   9,8  A
350       12   7,8    B
250       12   5,9      C

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Temperature   N  Mean  Grouping
90           18   8,6  A
80           18   7,1    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence
```

Time	N	Mean	Grouping
5	12	9,1	A
4	12	8,1	B
3	12	6,4	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	Temperature	N	Mean	Grouping
450	90	6	10,8	A
450	80	6	8,7	B
350	90	6	8,6	B
350	80	6	7,1	C
250	90	6	6,3	D
250	80	6	5,5	E

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	Time	N	Mean	Grouping
450	5	4	11,2	A
450	4	4	9,6	B
350	5	4	8,5	C
450	3	4	8,4	C
350	4	4	8,1	D
250	5	4	7,5	E
350	3	4	6,9	F
250	4	4	6,4	G
250	3	4	3,8	H

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Temperature	Time	N	Mean	Grouping
90	5	6	9,9	A
90	4	6	9,0	B
80	5	6	8,3	C
80	4	6	7,1	D
90	3	6	6,9	E
80	3	6	5,9	F

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	Temperature	Time	N	Mean	Grouping
450	90	5	2	12,2	A
450	90	4	2	10,9	B
450	80	5	2	10,2	C
350	90	5	2	9,5	D
450	90	3	2	9,3	D E
350	90	4	2	9,0	E

450	80	4	2	8,4	F
250	90	5	2	7,9	G
350	80	5	2	7,5	H
450	80	3	2	7,5	H
350	90	3	2	7,4	H I
350	80	4	2	7,3	H I
250	90	4	2	7,1	I
250	80	5	2	7,1	I
350	80	3	2	6,5	J
250	80	4	2	5,7	K
250	90	3	2	3,9	L
250	80	3	2	3,7	L

Means that do not share a letter are significantly different.

Table C. 17. Three-way ANOVA and Tukey's Comparison Test with 95% confidence level for pectin extraction yield of samples pressurized with acid on HHP assisted extraction

General Linear Model: Yield, % versus Pressure; Temperature; Time

Factor	Type	Levels	Values
Pressure	fixed	3	250; 350; 450
Temperature	fixed	2	80; 90
Time	fixed	3	3; 4; 5

Analysis of Variance for Yield, %, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Pressure	2	87,7838	87,7838	43,8919	6846,22	0,000
Temperature	1	19,9809	19,9809	19,9809	3116,60	0,000
Time	2	44,9580	44,9580	22,4790	3506,26	0,000
Pressure*Temperature	2	2,6225	2,6225	1,3112	204,53	0,000
Pressure*Time	4	5,9435	5,9435	1,4859	231,77	0,000
Temperature*Time	2	1,2426	1,2426	0,6213	96,91	0,000
Pressure*Temperature*Time	4	0,3589	0,3589	0,0897	14,00	0,000
Error	18	0,1154	0,1154	0,0064		
Total	35	163,0056				

S = 0,0800694 R-Sq = 99,93% R-Sq(adj) = 99,86%

Unusual Observations for Yield, %

Obs	Yield, %	Fit	SE Fit	Residual	St Resid
5	10,1100	10,2300	0,0566	-0,1200	-2,12 R
6	10,3500	10,2300	0,0566	0,1200	2,12 R
11	12,1000	12,2300	0,0566	-0,1300	-2,30 R
12	12,3600	12,2300	0,0566	0,1300	2,30 R

R denotes an observation with a large standardized residual.

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	N	Mean	Grouping
450	12	9,8	A
350	12	7,8	B
250	12	5,9	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Temperature	N	Mean	Grouping
90	18	8,6	A
80	18	7,1	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Time	N	Mean	Grouping
5	12	9,1	A
4	12	8,1	B
3	12	6,4	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	Temperature	N	Mean	Grouping
450	90	6	10,8	A
450	80	6	8,7	B
350	90	6	8,6	B
350	80	6	7,1	C
250	90	6	6,3	D
250	80	6	5,5	E

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	Time	N	Mean	Grouping
450	5	4	11,2	A
450	4	4	9,6	B
350	5	4	8,5	C
450	3	4	8,4	C
350	4	4	8,1	D
250	5	4	7,5	E
350	3	4	6,9	F
250	4	4	6,4	G
250	3	4	3,8	H

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Temperature	Time	N	Mean	Grouping
90	5	6	9,9	A
90	4	6	9,0	B
80	5	6	8,3	C
80	4	6	7,1	D
90	3	6	6,9	E
80	3	6	5,9	F

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	Temperature	Time	N	Mean	Grouping
450	90	5	2	12,2	A
450	90	4	2	10,9	B
450	80	5	2	10,2	C
350	90	5	2	9,5	D
450	90	3	2	9,3	D E
350	90	4	2	9,0	E

450	80	4	2	8,4	F
250	90	5	2	7,9	G
350	80	5	2	7,5	H
450	80	3	2	7,5	H
350	90	3	2	7,4	H I
350	80	4	2	7,3	H I
250	90	4	2	7,1	I
250	80	5	2	7,1	I
350	80	3	2	6,5	J
250	80	4	2	5,7	K
250	90	3	2	3,9	L
250	80	3	2	3,7	L

Means that do not share a letter are significantly different.

Table C. 18. Three-way ANOVA and Tukey's Comparison Test with 95% confidence level for pectin extraction yield of samples pressurized without acid on HHP assisted extraction

General Linear Model: Yield, % versus Pressure; Temperature; Time

```
Factor       Type   Levels  Values
Pressure     fixed       3  250; 350; 450
Temperature  fixed       2  80; 90
Time         fixed       3  3; 4; 5


Analysis of Variance for Yield, %, using Adjusted SS for Tests

Source                     DF    Seq SS   Adj SS   Adj MS        F      P
Pressure                    2   76,4600  76,4600  38,2300  6371,67  0,000
Temperature                 1   23,1361  23,1361  23,1361  3856,02  0,000
Time                        2   30,7321  30,7321  15,3660  2561,01  0,000
Pressure*Temperature        2    2,0835   2,0835   1,0417   173,62  0,000
Pressure*Time               4    5,8673   5,8673   1,4668   244,47  0,000
Temperature*Time            2    2,8361   2,8361   1,4180   236,34  0,000
Pressure*Temperature*Time   4    1,3351   1,3351   0,3338    55,63  0,000
Error                      18    0,1080   0,1080   0,0060
Total                      35  142,5581


S = 0,0774597   R-Sq = 99,92%   R-Sq(adj) = 99,85%


Unusual Observations for Yield, %

Obs  Yield, %      Fit  SE Fit  Residual  St Resid
 11   12,2000  12,0900  0,0548    0,1100      2,01 R
 12   11,9800  12,0900  0,0548   -0,1100     -2,01 R

R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95,0% Confidence

Pressure   N  Mean  Grouping
450       12   9,0  A
350       12   7,5    B
250       12   5,4      C

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Temperature   N  Mean  Grouping
90           18   8,1  A
80           18   6,5    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Time   N  Mean  Grouping
```

5	12	8,4	A
4	12	7,4	B
3	12	6,1	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	Temperature	N	Mean	Grouping
450	90	6	10,1	A
350	90	6	8,2	B
450	80	6	7,8	C
350	80	6	6,8	D
250	90	6	6,0	E
250	80	6	4,9	F

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	Time	N	Mean	Grouping
450	5	4	10,3	A
450	4	4	8,7	B
350	5	4	8,1	C
450	3	4	7,9	C
350	4	4	7,6	D
350	3	4	6,9	E
250	5	4	6,7	E
250	4	4	6,0	F
250	3	4	3,6	G

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Temperature	Time	N	Mean	Grouping
90	5	6	9,5	A
90	4	6	8,2	B
80	5	6	7,2	C
80	4	6	6,7	D
90	3	6	6,6	D
80	3	6	5,6	E

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	Temperature	Time	N	Mean	Grouping
450	90	5	2	12,1	A
450	90	4	2	9,5	B
350	90	5	2	9,0	C
450	90	3	2	8,8	C D
450	80	5	2	8,5	D E
350	90	4	2	8,3	E
450	80	4	2	8,0	F
250	90	5	2	7,5	G

350	90	3	2	7,4	G H
350	80	5	2	7,2	H I
450	80	3	2	7,0	I J
350	80	4	2	6,9	I J
250	90	4	2	6,8	J
350	80	3	2	6,4	K
250	80	5	2	5,9	L
250	80	4	2	5,2	M
250	90	3	2	3,7	N
250	80	3	2	3,5	N

Means that do not share a letter are significantly different.

Table C. 19. Three-way ANOVA and Tukey's Comparison Test with 95% confidence level for degree of esterification of samples pressurized with acid on HHP assisted extraction

General Linear Model: DE, % versus Pressure; Temperature; Time

```
Factor       Type   Levels  Values
Pressure     fixed       3  250; 350; 450
Temperature  fixed       2  80; 90
Time         fixed       3  3; 4; 5


Analysis of Variance for DE, %, using Adjusted SS for Tests

Source                      DF    Seq SS   Adj SS   Adj MS       F      P
Pressure                     2   20,6965  20,6965  10,3482   32,01  0,000
Temperature                  1   49,6555  49,6555  49,6555  153,61  0,000
Time                         2   25,6758  25,6758  12,8379   39,71  0,000
Pressure*Temperature         2    6,6107   6,6107   3,3053   10,22  0,001
Pressure*Time                4    5,6819   5,6819   1,4205    4,39  0,012
Temperature*Time             2    1,0847   1,0847   0,5423    1,68  0,215
Pressure*Temperature*Time    4    5,5237   5,5237   1,3809    4,27  0,013
Error                       18    5,8188   5,8188   0,3233
Total                       35  120,7476


S = 0,568565   R-Sq = 95,18%   R-Sq(adj) = 90,63%


Unusual Observations for DE, %

Obs    DE, %      Fit  SE Fit  Residual  St Resid
  5  39,0000  38,0000  0,4020    1,0000      2,49 R
  6  37,0000  38,0000  0,4020   -1,0000     -2,49 R
  7  34,0000  33,0000  0,4020    1,0000      2,49 R
  8  32,0000  33,0000  0,4020   -1,0000     -2,49 R

R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95,0% Confidence

Pressure   N  Mean  Grouping
250       12  36,6  A
350       12  35,6    B
450       12  34,8      C

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Temperature   N  Mean  Grouping
80           18  36,8  A
90           18  34,5    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence
```

Time	N	Mean	Grouping
5	12	36,6	A
4	12	35,8	B
3	12	34,6	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	Temperature	N	Mean	Grouping
250	80	6	37,2	A
350	80	6	37,0	A B
450	80	6	36,3	A B
250	90	6	36,1	B
350	90	6	34,1	C
450	90	6	33,3	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	Time	N	Mean	Grouping
250	4	4	37,6	A
250	5	4	37,3	A
350	5	4	36,7	A B
450	5	4	35,8	B C
350	4	4	35,3	B C D
250	3	4	35,0	C D
350	3	4	34,7	C D
450	4	4	34,6	C D
450	3	4	34,0	D

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Temperature	Time	N	Mean	Grouping
80	5	6	38,0	A
80	4	6	37,0	A
80	3	6	35,5	B
90	5	6	35,2	B
90	4	6	34,6	B C
90	3	6	33,6	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	Temperature	Time	N	Mean	Grouping
350	80	5	2	38,4	A
250	80	4	2	38,1	A B
450	80	5	2	38,0	A B
250	80	5	2	37,5	A B C
250	90	5	2	37,2	A B C D
350	80	4	2	37,0	A B C D

250	90	4	2	37,0	A B C D
450	80	4	2	36,0	B C D E
250	80	3	2	36,0	B C D E
350	80	3	2	35,5	C D E F
450	80	3	2	35,0	D E F G
350	90	5	2	35,0	D E F G
250	90	3	2	34,0	E F G
350	90	3	2	33,8	E F G
350	90	4	2	33,6	F G
450	90	5	2	33,5	F G
450	90	4	2	33,2	G
450	90	3	2	33,0	G

Means that do not share a letter are significantly different.

Table C. 20. Three-way ANOVA and Tukey's Comparison Test with 95% confidence level for degree of esterification of samples pressurized without acid on HHP assisted extraction

General Linear Model: DE, % versus Pressure; Temperature; Time

```
Factor       Type   Levels  Values
Pressure     fixed       3  250; 350; 450
Temperature  fixed       2  80; 90
Time         fixed       3  3; 4; 5


Analysis of Variance for DE, %, using Adjusted SS for Tests

Source                     DF   Seq SS   Adj SS  Adj MS       F      P
Pressure                    2   1,4361   1,4361  0,7180   12,30  0,000
Temperature                 1   7,9524   7,9524  7,9524  136,25  0,000
Time                        2  14,5801  14,5801  7,2900  124,90  0,000
Pressure*Temperature        2   1,7253   1,7253  0,8626   14,78  0,000
Pressure*Time               4   3,6809   3,6809  0,9202   15,77  0,000
Temperature*Time            2   4,1909   4,1909  2,0954   35,90  0,000
Pressure*Temperature*Time   4   3,7841   3,7841  0,9460   16,21  0,000
Error                      18   1,0506   1,0506  0,0584
Total                      35  38,4002


S = 0,241592   R-Sq = 97,26%   R-Sq(adj) = 94,68%


Unusual Observations for DE, %

Obs    DE, %      Fit  SE Fit  Residual  St Resid
 23  35,1900  34,6900  0,1708    0,5000      2,93 R
 24  34,1900  34,6900  0,1708   -0,5000     -2,93 R

R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95,0% Confidence

Pressure   N  Mean  Grouping
250       12  34,0  A
350       12  33,9  A
450       12  33,5    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Temperature   N  Mean  Grouping
80           18  34,3  A
90           18  33,4    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Time   N  Mean  Grouping
```

5	12	34,7	A
4	12	33,7	B
3	12	33,1	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	Temperature	N	Mean	Grouping
450	80	6	34,3	A
350	80	6	34,3	A
250	80	6	34,3	A
250	90	6	33,8	B
350	90	6	33,5	B
450	90	6	32,8	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	Time	N	Mean	Grouping
250	5	4	35,4	A
350	5	4	34,7	B
350	4	4	33,9	C
450	5	4	33,9	C
450	4	4	33,6	C D
250	4	4	33,6	C D
450	3	4	33,1	D
350	3	4	33,1	D
250	3	4	33,1	D

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Temperature	Time	N	Mean	Grouping
80	5	6	34,7	A
90	5	6	34,6	A B
80	4	6	34,2	B C
80	3	6	34,0	C
90	4	6	33,2	D
90	3	6	32,2	E

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	Temperature	Time	N	Mean	Grouping
250	90	5	2	36,2	A
450	80	5	2	34,7	B
350	90	5	2	34,7	B
350	80	5	2	34,7	B
250	80	5	2	34,7	B
450	80	4	2	34,3	B C
350	80	4	2	34,2	B C
250	80	4	2	34,1	B C

350	80	3	2	34,0		B	C	
250	80	3	2	34,0		B	C	
450	80	3	2	33,9		B	C	D
350	90	4	2	33,6			C	D
450	90	5	2	33,0				D E
250	90	4	2	33,0				D E
450	90	4	2	33,0				D E
450	90	3	2	32,4				E
350	90	3	2	32,2				E
250	90	3	2	32,1				E

Means that do not share a letter are significantly different.

Table C. 21. Three-way ANOVA and Tukey's Comparison Test with 95% confidence level for viscosity of samples pressurized with acid on HHP assisted extraction

General Linear Model: Viscosity versus Pressure; Temperature; Time

```
Factor       Type   Levels  Values
Pressure     fixed       3  250; 350; 450
Temperature  fixed       2  80; 90
Time         fixed       3  3; 4; 5


Analysis of Variance for Viscosity, using Adjusted SS for Tests

Source                     DF     Seq SS     Adj SS     Adj MS     F      P
Pressure                    2  0,0000001  0,0000001  0,0000000  9,90  0,001
Temperature                 1  0,0000000  0,0000000  0,0000000  8,77  0,008
Time                        2  0,0000000  0,0000000  0,0000000  6,89  0,006
Pressure*Temperature        2  0,0000000  0,0000000  0,0000000  0,64  0,541
Pressure*Time               4  0,0000000  0,0000000  0,0000000  0,94  0,464
Temperature*Time            2  0,0000000  0,0000000  0,0000000  0,51  0,611
Pressure*Temperature*Time   4  0,0000000  0,0000000  0,0000000  0,87  0,501
Error                      18  0,0000000  0,0000000  0,0000000
Total                      35  0,0000002


S = 0,0000506623   R-Sq = 74,24%   R-Sq(adj) = 49,90%


Unusual Observations for Viscosity

Obs  Viscosity       Fit    SE Fit   Residual  St Resid
 29   0,003990  0,004120  0,000036  -0,000130     -3,63 R
 30   0,004250  0,004120  0,000036   0,000130      3,63 R

R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95,0% Confidence

Pressure   N  Mean  Grouping
250       12   0,0  A
350       12   0,0    B
450       12   0,0    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Temperature   N  Mean  Grouping
80           18   0,0  A
90           18   0,0    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Time   N  Mean  Grouping
```

5	12	0,0	A
4	12	0,0	A B
3	12	0,0	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	Temperature	N	Mean	Grouping
250	80	6	0,0	A
250	90	6	0,0	A B
350	80	6	0,0	A B C
450	80	6	0,0	A B C
350	90	6	0,0	B C
450	90	6	0,0	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	Time	N	Mean	Grouping
250	5	4	0,0	A
350	5	4	0,0	A B
250	4	4	0,0	A B
250	3	4	0,0	A B
350	4	4	0,0	A B
450	5	4	0,0	A B
450	4	4	0,0	A B
450	3	4	0,0	B
350	3	4	0,0	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Temperature	Time	N	Mean	Grouping
80	5	6	0,0	A
80	4	6	0,0	A B
90	5	6	0,0	A B
90	4	6	0,0	B
80	3	6	0,0	B
90	3	6	0,0	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	Temperature	Time	N	Mean	Grouping
250	80	5	2	0,0	A
350	90	5	2	0,0	A B
350	80	5	2	0,0	A B
250	80	4	2	0,0	A B
250	80	3	2	0,0	A B
250	90	5	2	0,0	A B
450	80	5	2	0,0	A B
350	80	4	2	0,0	A B

250	90	4	2	0,0	A B
250	90	3	2	0,0	A B
450	80	4	2	0,0	A B
350	90	4	2	0,0	A B
450	80	3	2	0,0	B
350	80	3	2	0,0	B
450	90	5	2	0,0	B
450	90	4	2	0,0	B
350	90	3	2	0,0	B
450	90	3	2	0,0	B

Means that do not share a letter are significantly different.

Table C. 22. Three-way ANOVA and Tukey's Comparison Test with 95% confidence level for viscosity of samples pressurized without acid on HHP assisted extraction

General Linear Model: Viscosity versus Pressure; Temperature; Time

```
Factor       Type   Levels  Values
Pressure     fixed       3  250; 350; 450
Temperature  fixed       2  80; 90
Time         fixed       3  3; 4; 5


Analysis of Variance for Viscosity, using Adjusted SS for Tests

Source                     DF     Seq SS     Adj SS     Adj MS      F      P
Pressure                    2  0,0000000  0,0000000  0,0000000   5,20  0,016
Temperature                 1  0,0000001  0,0000001  0,0000001  39,37  0,000
Time                        2  0,0000000  0,0000000  0,0000000   0,36  0,704
Pressure*Temperature        2  0,0000000  0,0000000  0,0000000   0,83  0,452
Pressure*Time               4  0,0000000  0,0000000  0,0000000   0,78  0,554
Temperature*Time            2  0,0000000  0,0000000  0,0000000   1,37  0,278
Pressure*Temperature*Time   4  0,0000000  0,0000000  0,0000000   0,66  0,628
Error                      18  0,0000001  0,0000001  0,0000000
Total                      35  0,0000002


S = 0,0000531246   R-Sq = 77,11%   R-Sq(adj) = 55,50%


Unusual Observations for Viscosity

Obs  Viscosity       Fit    SE Fit   Residual  St Resid
 29   0,003910  0,003990  0,000038  -0,000080     -2,13 R
 30   0,004070  0,003990  0,000038   0,000080      2,13 R

R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95,0% Confidence

Pressure   N  Mean  Grouping
250       12   0,0  A
350       12   0,0  A B
450       12   0,0    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Temperature   N  Mean  Grouping
80           18   0,0  A
90           18   0,0    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Time   N  Mean  Grouping
```

3	12	0,0	A
4	12	0,0	A
5	12	0,0	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	Temperature	N	Mean	Grouping
250	80	6	0,0	A
350	80	6	0,0	A B
450	80	6	0,0	A B
250	90	6	0,0	A B C
350	90	6	0,0	B C
450	90	6	0,0	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	Time	N	Mean	Grouping
250	3	4	0,0	A
350	5	4	0,0	A
250	4	4	0,0	A
250	5	4	0,0	A
350	3	4	0,0	A
350	4	4	0,0	A
450	4	4	0,0	A
450	3	4	0,0	A
450	5	4	0,0	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Temperature	Time	N	Mean	Grouping
80	3	6	0,0	A
80	4	6	0,0	A
80	5	6	0,0	A B
90	5	6	0,0	B
90	3	6	0,0	B
90	4	6	0,0	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	Temperature	Time	N	Mean	Grouping
350	80	3	2	0,0	A
250	80	3	2	0,0	A
250	80	4	2	0,0	A
250	80	5	2	0,0	A
350	80	4	2	0,0	A
450	80	4	2	0,0	A
450	80	3	2	0,0	A
350	90	5	2	0,0	A

350	80	5	2	0,0	A
250	90	3	2	0,0	A
450	80	5	2	0,0	A
250	90	4	2	0,0	A
250	90	5	2	0,0	A
350	90	3	2	0,0	A
350	90	4	2	0,0	A
450	90	4	2	0,0	A
450	90	3	2	0,0	A
450	90	5	2	0,0	A

Means that do not share a letter are significantly different.

Table C. 23. Three-way ANOVA and Tukey's Comparison Test with 95% confidence level for galacturonic acid content of samples pressurized with acid on HHP assisted extraction

General Linear Model: Gal-A, % versus Pressure; Temperature; Time

```
Factor       Type   Levels  Values
Pressure     fixed       3  250; 350; 450
Temperature  fixed       2  80; 90
Time         fixed       3  3; 4; 5


Analysis of Variance for Gal-A, %, using Adjusted SS for Tests

Source                      DF   Seq SS   Adj SS  Adj MS       F      P
Pressure                     2  181,301  181,301  90,651  552,49  0,000
Temperature                  1   73,085   73,085  73,085  445,43  0,000
Time                         2   44,597   44,597  22,298  135,90  0,000
Pressure*Temperature         2   14,435   14,435   7,218   43,99  0,000
Pressure*Time                4    3,647    3,647   0,912    5,56  0,004
Temperature*Time             2    4,322    4,322   2,161   13,17  0,000
Pressure*Temperature*Time    4    2,093    2,093   0,523    3,19  0,038
Error                       18    2,953    2,953   0,164
Total                       35  326,433


S = 0,405065   R-Sq = 99,10%   R-Sq(adj) = 98,24%


Grouping Information Using Tukey Method and 95,0% Confidence

Pressure   N  Mean  Grouping
250       12  74,2  A
350       12  71,8    B
450       12  68,7      C

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Temperature   N  Mean  Grouping
80           18  73,0  A
90           18  70,1    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Time   N  Mean  Grouping
3     12  72,8  A
4     12  71,7    B
5     12  70,1      C

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence
```

Pressure	Temperature	N	Mean	Grouping
250	80	6	75,2	A
250	90	6	73,1	B
350	80	6	72,8	B
450	80	6	71,0	C
350	90	6	70,9	C
450	90	6	66,4	D

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	Time	N	Mean	Grouping
250	3	4	75,0	A
250	4	4	74,4	A
250	5	4	73,0	B
350	3	4	73,0	B
350	4	4	71,8	C
350	5	4	70,7	D
450	3	4	70,4	D
450	4	4	69,0	E
450	5	4	66,7	F

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Temperature	Time	N	Mean	Grouping
80	3	6	73,9	A
80	4	6	73,0	B
80	5	6	72,0	C
90	3	6	71,7	C
90	4	6	70,5	D
90	5	6	68,2	E

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	Temperature	Time	N	Mean	Grouping
250	80	3	2	75,9	A
250	80	4	2	75,1	A B
250	80	5	2	74,6	A B
250	90	3	2	74,2	A B C
350	80	3	2	73,9	B C
250	90	4	2	73,7	B C D
350	80	4	2	72,8	C D E
350	90	3	2	72,2	D E F
450	80	3	2	72,1	E F
350	80	5	2	71,7	E F
250	90	5	2	71,5	E F
450	80	4	2	71,1	F G
350	90	4	2	70,9	F G
450	80	5	2	69,8	G H
350	90	5	2	69,7	G H
450	90	3	2	68,8	H
450	90	4	2	66,8	I

450	90	5	2	63,5	J

Means that do not share a letter are significantly different.

Table C. 24. Three-way ANOVA and Tukey's Comparison Test with 95% confidence level for galacturonic acid content of samples pressurized without acid on HHP assisted extraction

General Linear Model: Gal-A, % versus Pressure; Temperature; Time

```
Factor       Type   Levels  Values
Pressure     fixed       3  250; 350; 450
Temperature  fixed       2  80; 90
Time         fixed       3  3; 4; 5


Analysis of Variance for Gal-A, %, using Adjusted SS for Tests

Source                      DF   Seq SS   Adj SS   Adj MS       F      P
Pressure                     2  186,120  186,120   93,060  609,61  0,000
Temperature                  1  126,638  126,638  126,638  829,57  0,000
Time                         2   52,210   52,210   26,105  171,01  0,000
Pressure*Temperature         2   35,797   35,797   17,899  117,25  0,000
Pressure*Time                4    1,581    1,581    0,395    2,59  0,072
Temperature*Time             2    2,954    2,954    1,477    9,68  0,001
Pressure*Temperature*Time    4    2,063    2,063    0,516    3,38  0,031
Error                       18    2,748    2,748    0,153
Total                       35  410,110


S = 0,390712   R-Sq = 99,33%   R-Sq(adj) = 98,70%


Grouping Information Using Tukey Method and 95,0% Confidence

Pressure   N  Mean  Grouping
250       12  76,9  A
350       12  74,8    B
450       12  71,4      C

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Temperature   N  Mean  Grouping
80           18  76,2  A
90           18  72,5    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Time   N  Mean  Grouping
3     12  75,8  A
4     12  74,5    B
5     12  72,8      C

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence
```

Pressure	Temperature	N	Mean	Grouping
250	80	6	77,8	A
350	80	6	76,3	B
250	90	6	76,0	B
450	80	6	74,6	C
350	90	6	73,3	D
450	90	6	68,1	E

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	Time	N	Mean	Grouping
250	3	4	78,0	A
250	4	4	77,0	B
350	3	4	76,4	B C
250	5	4	75,7	C D
350	4	4	75,2	D
350	5	4	72,9	E
450	3	4	72,9	E
450	4	4	71,3	F
450	5	4	69,9	G

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Temperature	Time	N	Mean	Grouping
80	3	6	77,3	A
80	4	6	76,3	B
80	5	6	75,1	C
90	3	6	74,2	D
90	4	6	72,7	E
90	5	6	70,6	F

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	Temperature	Time	N	Mean	Grouping
250	80	3	2	78,9	A
250	80	4	2	77,9	A B
350	80	3	2	77,4	A B C
250	90	3	2	77,2	B C D
250	80	5	2	76,6	B C D E
350	80	4	2	76,4	B C D E
250	90	4	2	76,1	C D E F
450	80	3	2	75,8	D E F
350	90	3	2	75,4	E F G
350	80	5	2	75,3	E F G
250	90	5	2	74,7	F G H
450	80	4	2	74,7	F G H
350	90	4	2	73,9	G H
450	80	5	2	73,4	H
350	90	5	2	70,6	I
450	90	3	2	70,0	I
450	90	4	2	68,0	J

450	90	5	2	66,4	K

Means that do not share a letter are significantly different.

Table C. 25. Three-way ANOVA and Tukey's Comparison Test with 95% confidence level for T2 of samples pressurized with acid on HHP assisted extraction

General Linear Model: T2 versus Pressure; Temperature; Time

```
Factor       Type   Levels  Values
Pressure     fixed       3  250; 350; 450
Temperature  fixed       2  80; 90
Time         fixed       3  3; 4; 5


Analysis of Variance for T2, using Adjusted SS for Tests

Source                      DF  Seq SS  Adj SS  Adj MS        F      P
Pressure                     2  346056  346056  173028  2621,68  0,000
Temperature                  1   34688   34688   34688   525,59  0,000
Time                         2  488655  488655  244327  3701,99  0,000
Pressure*Temperature         2   53132   53132   26566   402,52  0,000
Pressure*Time                4   54152   54152   13538   205,12  0,000
Temperature*Time             2   11888   11888    5944    90,06  0,000
Pressure*Temperature*Time    4    2932    2932     733    11,11  0,000
Error                       18    1188    1188      66
Total                       35  992691


S = 8,12397   R-Sq = 99,88%   R-Sq(adj) = 99,77%


Grouping Information Using Tukey Method and 95,0% Confidence

Pressure   N    Mean  Grouping
450       12  1189,3  A
350       12  1148,2    B
250       12   963,8      C

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Temperature   N    Mean  Grouping
90           18  1131,5  A
80           18  1069,4    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Time   N    Mean  Grouping
5     12  1252,0  A
4     12  1080,7    B
3     12   968,7      C

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence
```

Pressure	Temperature	N	Mean	Grouping
450	90	6	1205,6	A
450	80	6	1172,9	B
350	80	6	1155,2	C
350	90	6	1141,3	C
250	90	6	1047,5	D
250	80	6	880,2	E

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	Time	N	Mean	Grouping
450	5	4	1293,7	A
350	5	4	1281,6	A
250	5	4	1180,6	B
450	4	4	1174,8	B
350	4	4	1122,6	C
450	3	4	1099,4	D
350	3	4	1040,5	E
250	4	4	944,7	F
250	3	4	766,2	G

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Temperature	Time	N	Mean	Grouping
90	5	6	1282,9	A
80	5	6	1221,1	B
90	4	6	1089,6	C
80	4	6	1071,8	D
90	3	6	1022,1	E
80	3	6	915,3	F

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	Temperature	Time	N	Mean	Grouping
450	90	5	2	1307,0	A
350	90	5	2	1284,3	A B
450	80	5	2	1280,5	A B
350	80	5	2	1278,9	A B
250	90	5	2	1257,3	B
450	90	4	2	1183,4	C
450	80	4	2	1166,1	C
350	80	4	2	1163,2	C
450	90	3	2	1126,5	D
250	80	5	2	1103,9	D E
350	90	4	2	1081,9	E F
450	80	3	2	1072,2	E F
350	90	3	2	1057,8	F
350	80	3	2	1023,3	G
250	90	4	2	1003,4	G
250	80	4	2	886,1	H
250	90	3	2	881,9	H

250	80	3	2	650,5	I

Means that do not share a letter are significantly different.

Table C. 26. Three-way ANOVA and Tukey's Comparison Test with 95% confidence level for T2 of samples pressurized without acid on HHP assisted extraction

General Linear Model: T2 versus Pressure; Temperature; Time

```
Factor       Type   Levels  Values
Pressure     fixed       3  250; 350; 450
Temperature  fixed       2  80; 90
Time         fixed       3  3; 4; 5


Analysis of Variance for T2, using Adjusted SS for Tests

Source                     DF   Seq SS  Adj SS  Adj MS        F      P
Pressure                    2   412191  412191  206095  7797,55  0,000
Temperature                 1    83256   83256   83256  3149,98  0,000
Time                        2   388915  388915  194457  7357,23  0,000
Pressure*Temperature        2    11762   11762    5881   222,51  0,000
Pressure*Time               4    40193   40193   10048   380,17  0,000
Temperature*Time            2    56199   56199   28100  1063,14  0,000
Pressure*Temperature*Time   4    43149   43149   10787   408,13  0,000
Error                      18      476     476      26
Total                      35  1036141


S = 5,14109   R-Sq = 99,95%   R-Sq(adj) = 99,91%


Grouping Information Using Tukey Method and 95,0% Confidence

Pressure   N    Mean  Grouping
450       12  1162,6  A
350       12  1000,8    B
250       12   903,2      C

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Temperature   N    Mean  Grouping
90           18  1070,3  A
80           18   974,1    B

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Time   N    Mean  Grouping
5     12  1156,7  A
4     12  1006,4    B
3     12   903,6      C

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence
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Pressure	Temperature	N	Mean	Grouping
450	90	6	1189,8	A
450	80	6	1135,5	B
350	90	6	1046,7	C
250	90	6	974,4	D
350	80	6	954,9	E
250	80	6	831,9	F

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	Time	N	Mean	Grouping
450	5	4	1270,7	A
450	4	4	1124,4	B
350	5	4	1110,1	C
450	3	4	1092,8	D
250	5	4	1089,2	D
350	4	4	1023,4	E
250	4	4	871,2	F
350	3	4	868,8	F
250	3	4	749,1	G

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Temperature	Time	N	Mean	Grouping
80	5	6	1158,2	A
90	5	6	1155,2	A
90	4	6	1057,0	B
90	3	6	998,7	C
80	4	6	955,7	D
80	3	6	808,4	E

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95,0% Confidence

Pressure	Temperature	Time	N	Mean	Grouping
450	90	5	2	1305,3	A
450	80	5	2	1236,1	B
350	80	5	2	1147,6	C
450	90	4	2	1142,3	C D
450	90	3	2	1121,8	D E
450	80	4	2	1106,6	E F
250	80	5	2	1090,9	F G
250	90	5	2	1087,5	F G
350	90	5	2	1072,6	G H
450	80	3	2	1063,8	H
350	90	4	2	1055,2	H
350	90	3	2	1012,2	I
350	80	4	2	991,6	I J
250	90	4	2	973,5	J
250	90	3	2	862,2	K
250	80	4	2	768,9	L
350	80	3	2	725,5	M

250	80	3	2	635,9	N

Means that do not share a letter are significantly different.