Valorising Canadian cranberry (*V. macrocarpon*) pomace using microwave heating: recovery and physicochemical characterization of pectin

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To my mother, my father, and, more importantly, my Father.

To my beloved *trois sœur*.

PREFACE AND CONTRIBUTION OF AUTHORS

The present thesis has been written in accordance with the McGill University guidelines for thesis preparation. Specifically, the manuscript-based format was adopted throughout this thesis. It contains five chapters, some of which are published or prepared for publication as shown below:

- **Adetunji, L. R.**; A. Adekunle; V. Orsat; & V. Raghavan. 2016. "Advances in the pectin production process using novel extraction techniques: A review" (Accepted—in press).
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Lanrewaju Adetunji (main author) conducted the literature review, designed and performed all experiments, and wrote all the manuscripts. The research work was majorly conducted in the Postharvest Engineering laboratory, Department of Bioresource Engineering, Macdonald Campus of McGill University, Montreal.

Dr Vijaya Raghavan (co-author), James McGill Professor at the Bioresource Engineering department, McGill University, Macdonald Campus, Sainte-Anne-de-Bellevue, Quebec, supervised the research, provided scientific advice, and helped to revise the manuscripts. Mr Ademola Adekunle assisted in organizing and revising the review article for publication. Mr. Yvan Gariepy is an Academic Associate in the Department of Bioresource Engineering and facilitated the experimental works early on, as well as lending his technical expertise through the course of the research.

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ABSTRACT

Biomass consisting of cranberry (*Vaccinium macrocarpon* Aiton) pomace was evaluated as a potentially viable source of a poly-anionic functional polysaccharide (pectin). Using microwave (MW) intensification, the extraction process was optimized to enhanced yield. A probable standard variant of the process was also simulated at laboratory scale by conventional heating extraction in a thermostatic water bath. The physicochemical properties of recovered cranberry pectin (CBP) polysaccharide were characterized using a variety of chemical/structural, hydrodynamic, and rheological techniques, including structural assessment by FT-IR spectroscopy, quantification of main sugar units, antioxidant activity by UV/Vis spectroscopy, visual appearance by colour measurement, intrinsic viscosity and molecular weight determinations by viscometry, as well as flow and viscoelastic behaviour by rheometry.

Incorporating MW heating into the pectin extraction process resulted in: (a) at least 17% better recovery, (b) processing time by about 90%, (c) approximately 27% less solvent use; and (d) roughly 48% energy savings. The D-galacturonic acid (GalA) content of cranberry pectin obtained by both processes ranged from 43–52%, with extraction method significantly ($p < 0.05$) affecting GalA. The low methoxyl pectic polysaccharide had an intrinsic viscosity of 2.15 dl g^{-1} and viscosity-average molecular weight 39 kDa. Ostwald-de Waele's power-law model was sufficient to fit the flow data of 1.0–4.0 **wt%** CBP solutions, which all exhibit considerable shear-thinning behaviour. The 4.0 **wt%** solution was shown to display temperature-sensitive apparent viscosities. Employing Cox-Merz empirical rule revealed partial superimposability of steady shear and complex viscosity data for both 1.0 and 2.0 **wt%** CBP solutions, particularly at a mid (0.2-100 s^{-1} or rad s^{-1}) shear rate/angular frequency range; 4.0 **wt%** showed no such correlation.

Based on its demonstrated physicochemical properties, modes of potential functionalities of cranberry polysaccharide in food and related systems are submitted as viscosity enhancement and antioxidant dietary fibre, among others. This study, therefore, presents a potential opportunity for added income stream for cranberry juice processors from pomace.

RÉSUMÉ

Grâce à une intensification par micro-ondes, le rendement potentiel du processus d'extraction d'un polysaccharide fonctionnel poly-anionique (pectine) de la biomasse du marc de canneberge (*Vaccinium macrocarpon* Aiton) fut optimisé. Un procédé normalisé d'extraction thermique à l'échelle du laboratoire entrepris dans un bain-marie thermostatique servit de base de comparaison. Les propriétés physico-chimiques de la pectine de canneberge récupéré furent caractérisés par une variété de techniques chimiques/structurales, hydrodynamiques et rhéologiques : analyse structurale par spectroscopie infra-rouge par transformée de Fourier (IRTF), la quantification du résidu glucidique principal et de l'activité antioxydante par spectroscopie UV/Vis, apparence visuelle par colorimétrie, viscosité intrinsèque et poids moléculaire par viscosimétrie, ainsi que le flux et le comportement viscoélastique par rhéométrie.

L'incorporation du chauffage par micro-ondes dans le processus d'extraction de la pectine eut pour effet d'améliorer la récupération d'au moins 17%, de réduire le temps de traitement et l'utilisation de solvants de 90% et 27%, respectivement, donnant lieu des économies d'énergie d'environ 48%. Les études de caractérisation démontrèrent une teneur en acide D-galacturonique (GalA) de la pectine de canneberge variant entre 43% et 52% selon le procédé employé, la méthode d'extraction ayant un effet significatif ($p \leq 0{,}05$). Le polysaccharide pectique à basse teneur en méthoxyle montra une viscosité intrinsèque de 2,15 dl g^{-1} et une masse moléculaire moyenne déterminée par la viscosité de 39 kDa. Un modèle à base de loi de puissance Ostwald-de-Waele s'avéra s'ajuster adéquatement aux les données de flux de solutions de pectine de canneberge de 1,0 à 4,0% au poids, qui présentèrent tous un comportement rhéofluidifiant considérable. La viscosité apparente de la solution de pectine de canneberge à 4,0% s'avéra thermosensible. L'emploi de la règle empirique Cox-Merz révéla une superposition partielle du cisaillement constant et des données de viscosité complexes pour les solutions de pectine de canneberge de 1.0 et 2.0% au poids; particulièrement à des taux de cisaillement/fréquence angulaire moyens (0.2-100 s^{-1} ou rad s^{-1}). Cette superposition n'exista pas pour les solutions de 4,0% au poids.

Étant donné leurs propriétés physico-chimiques définies ci-haut, les polysaccharides de canneberge utilisés comme ajouts aux aliments et systèmes connexes peuvent fonctionner pour améliorer la viscosité et fournir des fibres alimentaires antioxydants, entre autres. Cette étude souligne le potentiel d'un revenu supplémentaire pour les transformateurs de jus de canneberges, axée sur le marc de canneberges.

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Lanre,

May, 2016

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ABBREVIATIONS AND LIST OF SYMBOLS

Abbreviations

	Full form
AA	antioxidant activity
ANOVA	analysis of variance
AOAC	Association of Official Analytical Chemists
BBD	Box-Behnken design
CB	cranberry
CBP	cranberry pectin
CHE	conventional heating extraction
CIELAB	*L*a*b** colour space by commission internationale de l'éclairage
CM	casein micelles
DA	degree of amidation
DAc	degree of acetylation (acetyl esterification)
db	dry weight basis
DE	degree of esterification (*compare DM*)
DM	degree of methyl esterification (*compare DE*)
DP	degree of polymerization
DPPH*	diphenylpicrylhydrazyl
EAE	enzyme-assisted extraction
EC	European Commission
FAO	Food and Agricultural Organisation of the United Nations
FDA	United States Food and Drug Administration
FT-IR	Fourier transform infrared spectroscopy
GalA	α-D-galactopyranosyluronic acid
GRAS	generally recognized as safe
HG	homogalacturonan
HM	high methoxyl
IR	infrared
LLE	liquid-liquid extraction
LM	low methoxyl (methyl ester)
LS	solvent to biomass ratio or liquid to solid ratio (*also L/S or L:S*)

LVE	linear viscoelastic
LVR	linear viscoelastic region
MAE	microwave-assisted extraction
MW	microwave
MH	Mark-Houwink equation (*also MHKS and MHS*)
MHKS	Mark-Houwink-Kuhn-Sakurada
MHS	Mark-Houwink-Sakurada equation
NS	neutral sugar
NSP	non-starch polysaccharide
OD	osmotic distillation
PEF	pulsed electric field
PSD	particle size distribution
RCF	relative centrifugal force
RG	rhamnogalacturonan
Rha	L-rhamnose
RI	refractive index
RO	reverse osmosis
RSM	response surface methodology
SAS	statistical analysis software
SAOS	small amplitude oscillatory shear
SDC	sweetened dried cranberry
SG	substituted galacturonan
S/L	solid to liquid ratio or biomass to solvent ratio (*compare LS*)
SLE	solid-liquid extraction
SWE	subcritical water extraction
TPC	total phenolic content
UAE	ultrasound-assisted extraction
USB	universal serial bus
USDA	United States Department of Agriculture
UV-Vis	ultraviolet-visible light spectroscopy
v/v	volume by volume
v/w	volume by weight
WHO	World Health Organization of the United Nations
w/v	weight by volume

w/w	weight by weight
wb	wet weight basis
XG	xylogalacturonan
ZSV	zero-shear viscosity

Symbols

Symbol	***Quantity***	***Unit***
a	MHKS parameter	
c	concentration	g ml^{-1}
CBP	cranberry pectin yield	mg g^{-1} of dry pomace
c_m	Fedors' concentration limit	g ml^{-1}
D_{eff}	diffusion coefficient	m^2 s^{-1}
E_a	flow activation energy	J mol^{-1}
f	frequency	Hz
G'	storage modulus	Pa
G''	loss modulus	Pa
$\|G^*\|$	absolute value of complex modulus	Pa
K	MHKS parameter	dl g^{-1}
K_H	Huggins' constant	
K_K	Kraemer's constant	
LS	solvent-biomass ratio	ml g^{-1}
m	mass	kg or g
M_n	number-average molecular weight	Da or g mol^{-1}
M_v	viscosity-average molecular weight	Da or g mol^{-1}
M_w	weight-average molecular weight	Da or g mol^{-1}
pH	solvent acidity	
R_{ED}	ratio of absorbance peak area correlating with DE	
T	temperature	°C or K
$time$	microwave irradiation time	min

Greek letters

Letter	***Quantity***	***Unit***
γ	strain	
$\dot{\gamma}$	shear rate	s^{-1}

δ	phase angle	
ε'	dielectric constant	
ε''	dielectric loss factor	
$[\eta]$	intrinsic viscosity	dl g^{-1}
η	apparent viscosity of solvent	
η_∞	infinite shear rate viscosity	Pa.s
η_0	zero shear rate viscosity	Pa·s
η_{inh}	inherent viscosity	dl g^{-1}
η_s	apparent solution viscosity	Pa·s
η_r	relative viscosity	
η_{red}	reduced viscosity	dl g^{-1}
ρ	density	kg m^{-3}
τ	shear stress	Pa
υ	kinematic viscosity	m^2 s^{-1}
ω	angular frequency	rad s^{-1}
ω_c	crossover angular frequency	rad s^{-1}

1. INTRODUCTION

1.1 Background

The food industry faces a myriad of challenges, both on the process and product fronts. The urgency to remedy these numerous problems is in fact matched by the increasingly demanding and enlightened consumers; this then leads to an increased involvement of regulatory bodies towards ensuring due diligence in the food industry operations. One of such challenges has been the question of waste handling and management. The food supply chain has been particularly pointed out as being inefficient, due to its production and accumulation of large volumes of wastes (in all three forms of matter) (Pfaltzgraff *et al.*, 2013). The statistics on these wastes are quite staggering, with one commonly cited Food and Agriculture Organization (FAO) estimate putting the disuse at around 1/3 of initial production (that is a futile third of water, land, and human resource inputs). But growth in the perception of the diversity of chemical constituents inherent in these side-streams, coupled with the move towards lowering the environmental (carbon) footprint of the agro-food sectors, has led to a teeming area of investigation—the valorisation of food supply chain wastes. In addition, concerns over the depletion of the world's fossil resources, has contributed to this 'bio-based' drive in no little way.

Numerous approaches are available by which transformation and valorisation are instilled into these food processing wastes (mostly plant-based, but also animal, wastes), namely chemical and bio-catalysis, bioconversion, clean synthesis, biofuels generation, among many others (Lin *et al.*, 2013). However, it appears that, with very few exceptions, an extraction/separation of naturally synthesized chemicals (such as starch, cellulose, chitosan, phytochemicals, hemicellulose, pectin, polyphenols, resins, sugars, amino acids, collagen, bacterial polysaccharides, etc.) from the less valuable constituents is always preliminary in the downstream conversions of these biomasses. Extracted molecules may be used as food additives or for exerting peculiar effects on human health (Ames, 1983). Often times, these biologically active (bioactive) compounds are extracted from such food processing by-products that would otherwise have gone into such less-rewarding uses as animal fodder or even as environmental pollutants in landfills.

Yet it is in solving one problem that another arises. The traditional processes for the extraction of valuable compounds from food processing wastes are not only time-, solvent-, and energy-

intensive, but can also be environmentally invasive in a way that contravenes its original purpose. 'Benign by design' can be regarded as the core tenet of the 'green chemistry' movement that started in the early 1990's. Its advent not only sought to ensure efficient utilization of resources and the affiliated minimization of waste, but also to address the environmental, health, and safety issues involved in the handling of chemical products—that is, pollution prevention through waste minimization as against an end-of-pipe solution of waste removal is advocated (Clark *et al.*, 2014). Additionally, among the key thrusts of green chemistry is the design of environmentally friendly solvents (Anastas and Kirchhoff, 2002). Organic solvents are widely used in the chemical, food and pharmaceutical industries in carrying out a number of operations including synthesis, processing, and separation. However, large volumetric requirements, coupled with extended time needed for their use in such operations which results in degradation of solutes, means that the continued use of these solvents is not economical. This is particularly so apparent in separation (including extraction) processes.

Another challenge today's food industry faces is the need to exceed the very high expectation of the health-conscious consumers for cheaper, more palatable and convenient foods, which are also nutritious and healthy (Norton, Fryer, and Moore, 2006). These days, such consumers are only willing to expend their disposable incomes on foods that provide the gratifying sensation of fatty foods without an actual excess fat content. Food industry practitioners and scientists, are tackling this challenge by adopting a structure–function approach towards identifying products and implementing processes that can simulate the texture of fat. A case in point is the acknowledgement, by manufacturers and researchers, that high molecular weight food polymers, among other qualities, are acquiescent to maintaining the food texture while minimizing or eliminating actual fat content in food (Lillford and Norton, 1994). Interestingly, many of these biopolymers (collectively regarded as hydrocolloids) are present in the wastes generated by the agro-food industry. Considering the entire agro-food industry as a closed system makes it possible to internally solve a number of its problems. **Figure 1.1** is a schematic depiction of such a sustainable closed system.

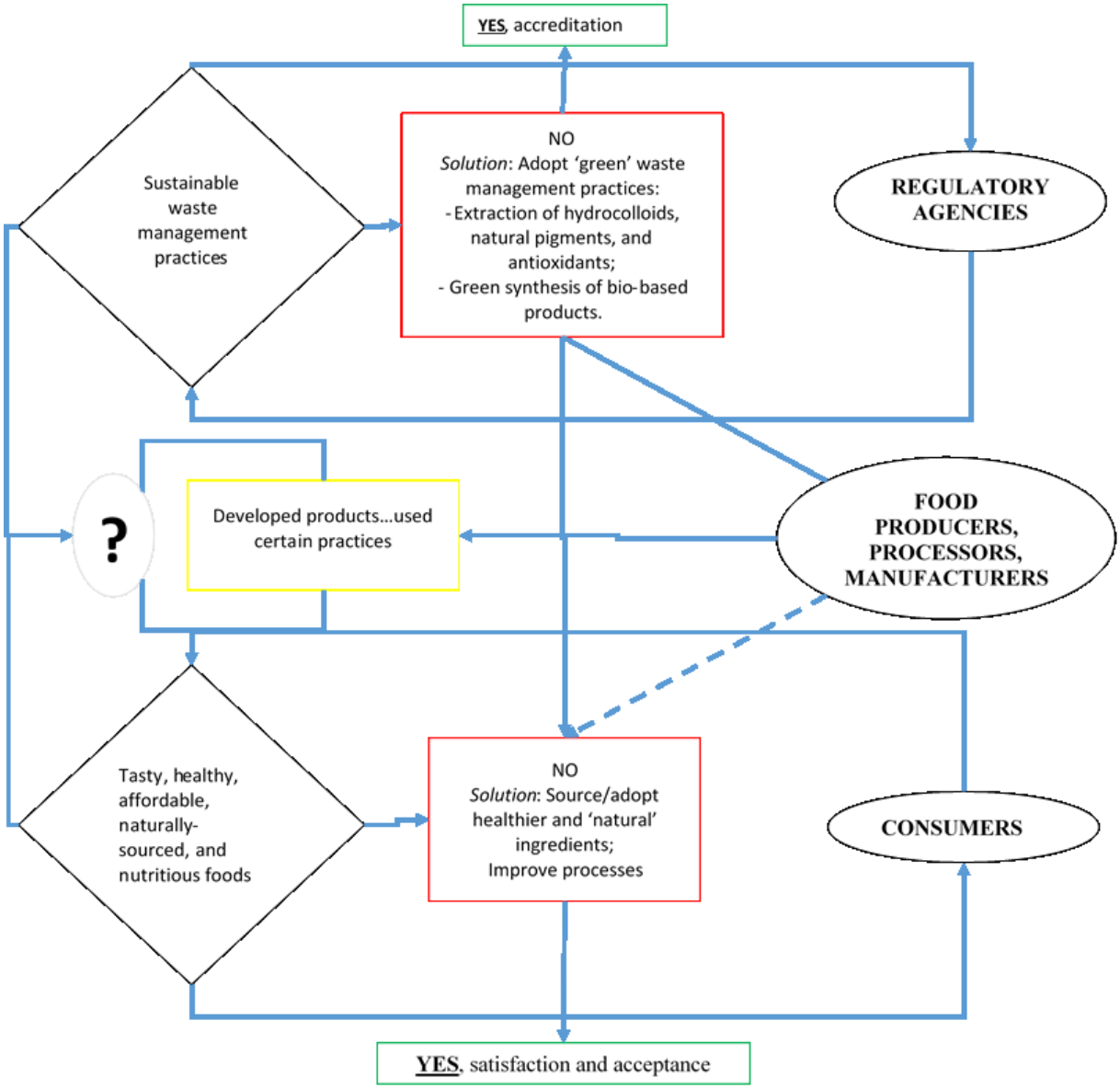


Figure 1.1. The framework of the challenge–solution approach towards agro-food industry sustainability and profitability.

A quintessential example of such recovered compounds from food waste is pectin, a complex hetero-polysaccharide found in the cell wall and middle lamella of many higher plants, mostly fruits and vegetables, where they act as hydrating and cementing agents (Thakur *et al.*, 1997). With an estimated annual worldwide consumption of about 45 million kilograms in 2003 (Savary *et al.*, 2003), pectin is a commercial food additive with no record of toxicity. Hence, its acceptable daily intake (ADI) is documented as "not specified" in the report of the 21st Joint FAO/WHO Expert Committee on Food Additives (JECFA) (WHO, 1981). Furthermore, it is a 'generally recognized as safe' (GRAS) ingredient in food products by the Food and Drug Administration (FDA) (Wang, Pagán, and Shi, 2002). Consequently, applications of pectin

have been reported in the food, pharmaceutical & cosmetic industries (Lupi *et al.*, 2015), and increasingly in the recent past, in the bio-based polymer research and industry (Fabra, López-Rubio, and Lagaron, 2014). Its use, both singly and in combination with other biopolymers, in the synthesis of drug-delivery systems is a typical example of the bio-based industry application (Liu, Fishman, and Hicks, 2007). At the core of some of these application is the gel-forming ability of pectin under certain conditions. Nonetheless, many food and other related uses of pectin stem from its thickening, stabilizing and emulsifying abilities (Lopes da Silva and Rao, 2006).

Pectin is ubiquitous in the tissues of fruit and vegetable, and the commercial sources of the compound include apple pomace, citrus peel, and to a lesser extent, sugar beet pulp—that are the by-products of the fruit juicing and vegetable production industries (Thakur *et al.*, 1997). In addition to the quality requirements, another factor influencing industrial use of a pectin-containing raw material is its availability in sufficient quantity (Thakur *et al.*, 1997). Primed by the increasing market demands, various sources of pectin and other hydrocolloids, including onion, sunflower, peach, pumpkin, pomegranate, kiwifruit, mango, rapeseed, soy processing wastes, etc., have been investigated over the years (Alexander and Sulebele, 1973; Matora *et al.*, 1995; May, 1990; Miyamoto and Chang, 1992; Moorthy *et al.*, 2015; Pagán *et al.*, 2001; Ptichkina, Markina, and Rumyantseva, 2008). However, beside a study by Baker and Kneeland (1936), investigations on assessing the suitability of cranberry pomace for pectin recovery are almost non-existent.

Cranberry (*Vaccinium macrocarpon*) is a major commercial crop native to and mainly cultivated in the U.S. (states of Massachusetts, New Jersey, Oregon, Washington, and Wisconsin), and Canada (British Columbia and Quebec provinces) with over 527 thousand metric tonnes of the fruit produced in 2013 (FAOSTAT). Chile is the third largest producer of cranberries. The most common processing of cranberries in Canada involves juice concentrate production, from which large amounts of the solid by-product are then discarded. Cranberry pomace—the solid by-product of its juicing industry—has been extracted for pigments and phenolic compounds (Park and Zhao, 2006; Zheng and Shetty, 1998). However, even with such uses available, a lot of cranberry pomace still go to waste, in the form of soluble and insoluble fibres. Being a fruit biomass, cranberry pomace could be utilized as an alternative pectin source, for additional income generation for the processors, as well as for the development of a new industry. An enquiry into the characteristics and structure of the pectin from cranberry is required towards valorising this acidic waste stream and to classify it towards downstream

functionalities.

Pectin in native biomass solubilizes in hot aqueous mineral acids (pH ~2) (Wang *et al.*, 2002). The conventional method of extracting pectin is equally solvent-intensive and requires extended heating period, making it time- and energy-consuming. Therefore, among the entire pectin production stages, the extraction operation has been identified as the main bottleneck (Casas-Orozco *et al.*, 2015). In view of expediting the mass transfer events, numerous investigations have been conducted using alternative extraction techniques, specifically: the use of microwave energy (Fishman and Chau, 2000; Fishman *et al.*, 2006), ultrasound pulses (Bagherian *et al.*, 2011), subcritical fluid (Ueno *et al.*, 2008), and enzyme augmentation (Ptichkina *et al.*, 2008) of existing methods, among others. However, as has been shown in prior studies, improper control of the process conditions and parameters in the novel extraction technologies can actually be counterproductive. It can lead to altering the molecular structure, to a degree that diminishes the rheological functionality of the resulting pectin (Wang *et al.*, 2002; Zhang *et al.*, 2013). For this reason, statistical and mathematical process optimization methodologies (such as response surface methodology and artificial neural networks) have been used to improve the extraction techniques.

1.2 Hypothesis

Based upon substantiated evidence in literature regarding the suitability of microwave energy in extracting high value compounds, it is herein hypothesized that the microwave-assisted extraction can be used for the efficient recovery of pectic polysaccharides from cranberry pomace. In particular, when the non-enzymatic approaches are adopted for the juicing process, the pectin of the unused biomass remains intact. Furthermore, it is hypothesized that the structural and functional properties of the extracted pectin can potentially serve a number of food production and processing needs.

1.3 Rationale

The high environmental footprint and the large handling cost, encountered as an aftermath of unutilized by-products of food processing industries are increasingly noticeable. Identifying and targeting high-value products in side streams can end the current practice of fruit and vegetable processors paying a levy to landfill their waste. More so, extracting these compounds can result in additional income generation for these processors. The microwave-assisted extraction (MAE) technique, despite higher initial cost, has proven useful in saving time and energy. Therefore, if adopted by the fruit and vegetable processors for the extraction of a

number of compounds, MAE can deliver many of these benefits.

1.4 Research objectives

The overall objective of this research is to optimize the extraction of pectin from cranberry and to characterize the recovered polysaccharide. These objectives entail the following sub-objectives:

1. Microwave-assisted extraction of cranberry pomace pectin will be optimized using response surface methodology (RSM), while comparing results with the conventional pectin extraction method.
2. Understand the chemical, macromolecular, and rheological properties of cranberry pectin to ascertain its suitability for potential end-use(s).

2. REVIEW OF LITERATURE

2.1 Cranberry processing and by-products

This section briefly discusses the production of cranberry and the size of the cranberry market. The common processing routes of the fruit, as well as by-products from such processes are also highlighted. It also presents an overview of the common (and prospective) utilization routes for these side streams.

2.1.1 Cranberries: production, processing and processing side streams

Taxonomically, cranberry is classified as family, *Ericaceae*; subfamily, *Vacciniaceae*; genus, *Vaccinium*; and the subgenus, *Oxycoccus*. The Ericaceae family are a morphologically diverse (including herbs, dwarf shrubs, shrubs, and trees) range of plant taxa but mainly consisting of wooded shrubs that are commonly found growing in acidic, infertile growing conditions. Common species of cranberries that have been identified include: *oxycoccus*, *microcarpum*, *macrocarpon*, and *erythrocarpun*. In North America, *Vaccinium macrocarpon* (large cranberry) is considered by the U.S. Department of Agriculture (USDA) as the standard crop for both fresh consumption and for juice cocktail. Conversely, *V. oxyccos* is the cultivated variety of cranberry in Germany, Finland and certain areas of Central Europe where it is usually referred to as lignoberry or English mulberry (Girard and Sinha, 2006).

2.1.1.1 World production of cranberries

Cultivation

Cranberries are cultivated as vines growing on bed layered with sand, peat, and gravel, which are traceable to previous glacial deposits (Girard and Sinha, 2006). Moreover, it is a common practice that growers do not replant the crop yearly as undamaged cranberry vine will survive indefinitely. This point is illustrated by certain vines that have been producing berries for over hundred years (Alston, Medellín-Azuara, and Saitone, 2014). The acreage for cranberry cultivation in the US was about 40,000 in 2014 (USDA-NASS, 2015).

Harvesting

Generally, cranberry is a Fall-blooming crop, with harvesting done typically between September and early November (Alston *et al.*, 2014; Girard and Sinha, 2006). One factor that informs readiness for harvest is the colour, which is influenced by the growing region and the variety of the crop.

As Girard and Sinha (2006) pointed out, there are two common routes of harvesting

cranberries, with a number of modifications having been made over the years. Additionally, the mode of harvesting chosen is dependent on the cranberry market targeted. Perhaps the earliest method of harvesting cranberry is known as the "dry harvest" method. In the past, it involved manually scooping cranberries off the vines. More recently, though, mechanical pickers, equipped with moving metal teeth, have been designed and developed. The "wet harvest" method utilizes the aero- and hydro-dynamic properties of cranberries to effect harvesting. Since fresh cranberry has air pockets inside it, the crop field will be flooded to cause the berries to float in water. To further aid in wet harvesting, water reels—known as egg-beaters—are used to detach the cranberries from their vines through a stirring action. The floating fruits are then enclosed in a corral using 'booms' where they can be vacuumed into trucks for transportation to the receiving stations for further processing. Hence, while dry-harvested cranberries mostly serve the fresh fruit market for use in cooking and baking, most wet-harvested cranberries are used for juice, sauces and as ingredients in processed food systems (Girard and Sinha, 2006).

Consumption

In 2013, worldwide production of cranberries was about 540 thousand metric tonnes according to the UN's FAO database (FAOSTAT, 2015). Of that number, over 97% was produced in Canada (mainly in the Quebec and British Columbia provinces) and the U.S. (mainly Wisconsin, Massachusetts, New Jersey, Oregon, and Washington). The crop is processed and marketed by U.S. and Canadian processors and handlers into four major categories: fresh cranberries, sauce products, sweetened dried cranberries (SDCs), and concentrates (including juice and juice drink products) (Alston *et al.*, 2014).

Ingesting cranberry has been reported as preventing urinary tract infections (Grace *et al.*, 2012). As a longstanding North American tradition, cranberry sauces are served in Thanksgiving dinners (Whitman-Salkin, 2013).

2.1.1.2 Chemical composition

Table 2.1 presents the general chemical composition of two species (*V. macrocarpon and V. oxycoccus*) of cranberries (Česonienė and Daubaras, 2015; Eck, 1990; Liebster, 1972). As is characteristic of vegetables and fruits, cranberries contain high amount of moisture; the smaller *V. oxycoccus* can have slightly higher percentage moisture (between 84.2 and 92%) than the larger macrocarpon (up to 88%). The pectin content, as a percentage of cranberry wet weight, ranges from 0.2–1.4%.

Table 2.1. Proximate chemical composition of two main species of cranberry

Constituent	Large cranberry, (*Vaccinium macrocarpon*)	Small cranberry, (*Vaccinium oxycoccos*)
Moisture, %	84.8–88.0	84.2–92.0
Dry matter, %	9.2–15.2	8.0–15.1
Mineral content, %	0.2–0.23	0.2–0.28
Titratable acidity, %	1.9–2.4	2.1–4.9
Monosaccharides, %	3.4–7.1	2.2–6.0
Sucrose, %	3.9–5.3	0.1–1.57
Fructose, %	1.0–2.2	1.0–2.2
Pectins, % (wb)	0.4–1.3	0.2–1.4
Benzoic acid, mg/100g	65.0–123.0	41.0–63.4
Ascorbic acid, mg/100g	7.5–32.1	15.3–30.0

2.1.1.3 Industrial processing of cranberry

A 2011 report by the National Agricultural Statistics Service division of the USDA (USDA-NASS, 2015) indicated that about 97 percent of the cranberries produced in the five producing states in the U.S. was processed in some way. A considerable range of products are obtained from cranberry industrially (**Fig. 2.1**). Although cranberries are mostly processed into fruit beverages—especially cranberry juice—in the United States and Canada, a significant percentage of these berries are also used as ingredients in food products including purees, pulps, SDCs, bakery and confectionary additives, fruit nectars, nutraceutical powder, jam, jellies and marmalades. Other common processed products that incorporate cranberries include dairy products like yogurts, milk-fruit desserts, and alcohol-free milk beverages (Cano, 2008; Pátkai, 2006). The process flows for some of these products are shown in **Figure 2.2**.

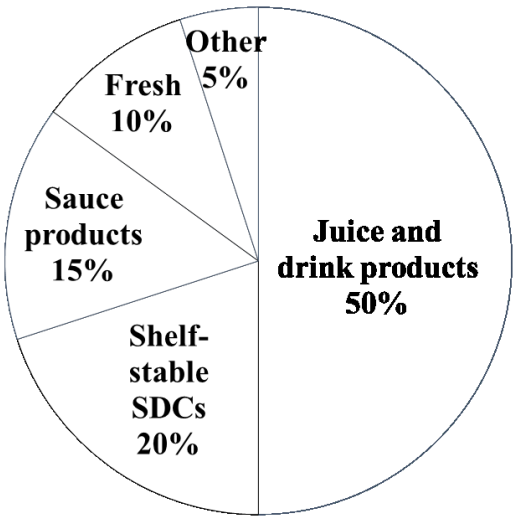


Figure 2.1. Utilization routes of cranberry [Data adapted from Girard and Sinha (2006)].

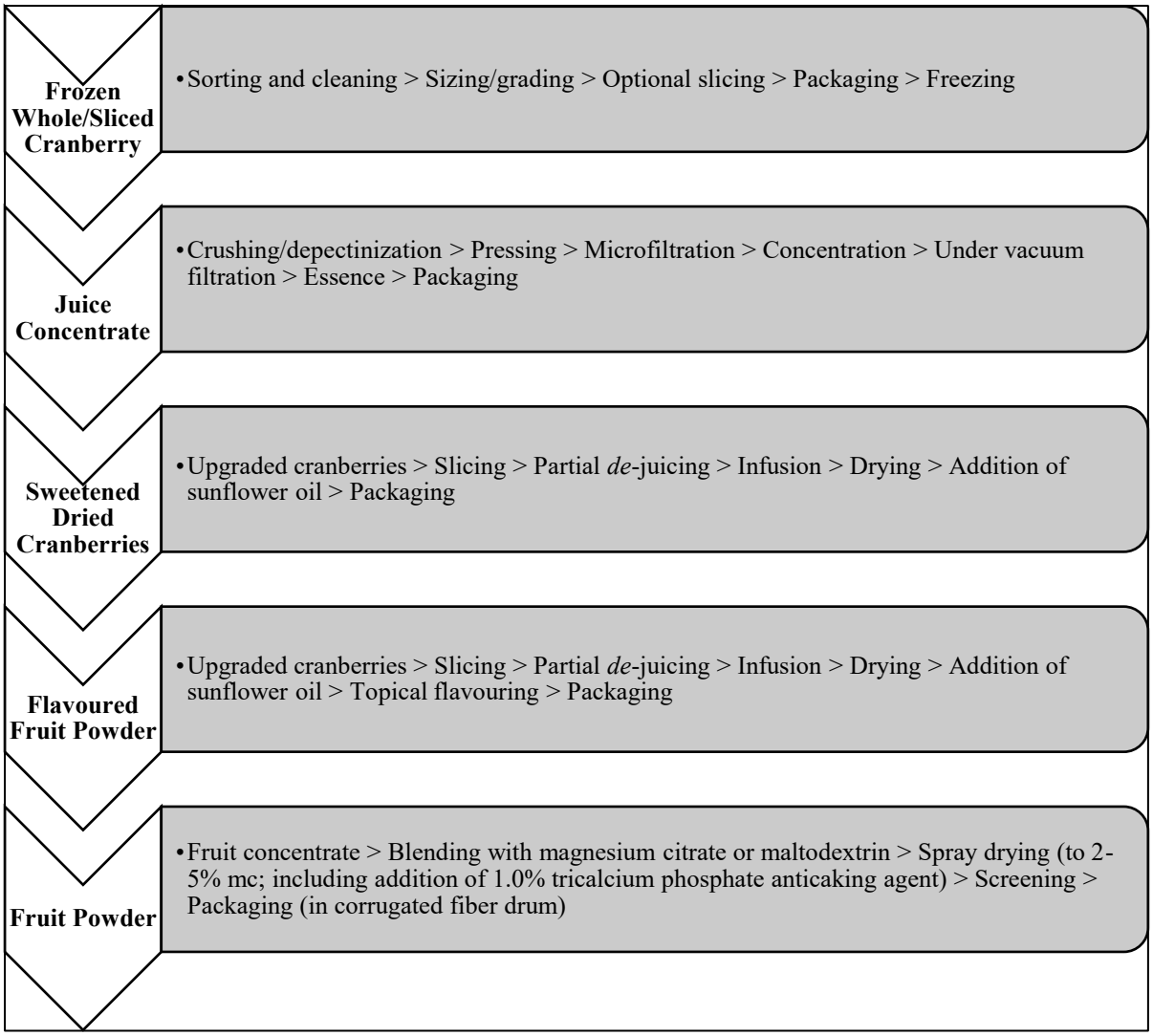


Figure 2.2. The process flow for cranberry products [Adapted from Girard and Sinha (2006)].

Juice

As mentioned before, juicing represents the most important route of obtaining processed cranberry. In the Codex Alimentarius, fruit juice is defined as "*the unfermented but fermentable liquid obtained from the edible part of sound, appropriately mature and fresh fruit or fruit maintained in sound condition by suitable means including post-harvest surface treatment applied in accordance with the applicable provisions of the Codex Alimentarius Commission*" (Bates, Morris, and Crandall, 2001; FAO/WHO, 2005). Freshly harvested cranberries are cleaned, sorted by colour, and frozen for subsequent use. As the need arises, the frozen cranberries are then extracted, filtered and concentrated to about 50°Brix juice concentrate (Girard and Sinha, 2006).

Juice extraction from the cranberries, through the application of mechanical pressure, follows

one of three distinct routes: ‘pressing’, ‘mash depectinization’ and ‘counter-current extraction’ (**Table 2.2**). Also, although mash depectinization is often favoured due to its comparatively higher yield, long process duration at elevated temperatures and the attendant off-colour and off-flavour of the resultant juice means that many other processors still opt for the non-enzymatic routes (i.e., pressing and counter-current extraction). Filtration follows one of the above-listed extraction techniques. This operation can be performed with filters such as plastic or stainless steel screens, woven fibre bags, media like diatomaceous earth and perlite, or polymer and ceramic membranes. Pore sizes of filters used in this step range from nanofilter (0.001–0.008 μm) to media (< 5 μm) (Girard and Sinha, 2006).

Filtered cranberry juice is then concentrated into a standard concentrate of around 50°Brix. This concentration step is beneficial in many sense, including improvement of microbial stability and volume reduction; and while the former helps extend product shelf life, the latter results in significant storage and shipping cost savings (Zambra *et al.*, 2015). Nevertheless, since cranberry juice contains sensory compounds that are especially temperature-labile, the concentration operation has to be performed with minimal loss of these healthful components. Several techniques, or their combinations, are used to produce fruit concentrates. These include evaporation, freezing and reverse osmosis (RO). However, it is a common industrial practice to combine RO with evaporation, in which case, the initial application of RO, which involves increased osmotic pressure, concentrates the fluid up to 30 °Brix, thus serving as a pre-concentration step before evaporation is applied to further raise the Brix level (Girard and Sinha, 2006). In recent times, a rather novel process known as osmotic distillation, featuring an osmotic gradient under isothermal conditions, is being increasingly applied for the concentration of fruit juices, and indeed other liquid foods such as milk, a situation largely driven in particular by the method’s mildness towards heat and shear labile compounds (including macro- and microsolutes) as well as its rapidness (Hogan *et al.*, 1998; Zambra *et al.*, 2015). Its applicability has been successfully demonstrated in the cranberry juice concentration process (e.g., °Brix increase from 8.6 to 48 °Brix is achievable in 18 min), with reported preservation of phenolic compounds, especially anthocyanins (Zambra *et al.*, 2015).

Table 2.2. Common cranberry juice extraction methods.

Juicing method	*Pressing*	*Mash depectinization*	*Counter-current extraction*
Principle/process conditions	Giant Press and Mechanical expression	Enzymes; Bucher Press or Centrifugation	Screw press
Advantages	Relatively cold process results in high-quality juice with excellent flavour	High yield	Relatively high yield; gentle process results in high quality juice; co-product with the juice can be processed into SDCs; intact cell soluble fibre including pectin.
Disadvantages	Low yield due to pectin and other fibre network preventing further extraction	Slightly elevated temperatures (around 52 °C) and long duration (about 4–12 h) required for depectinization results in degradation which alters the juice colour, flavour and shelf-life	Yields not as high as in mash depectinization.
Juice Yield	Approximately 75%	Can be over 100%	90%

Downstream commercial conversion of cranberry juice concentrates then takes one of two forms. "Cranberry juice cocktail" are obtained from *re*-diluted and sweetened concentrates. Concentrates are also blended with other fruit juices to reduce tartness (Grace *et al.*, 2012).

2.1.2 Handling and utilization of cranberry juice processing waste

Evidently, not all of the starting raw material introduced into fruit processing is converted into juice; upon obtaining the finished products, a large volume of the input fruits become by-products which are not suitable for human consumption, mainly due to their inferior nutritive content. Cranberry processing exemplifies this point. Recovering this processing waste happens either in the form of wastewater or solid pomace—consisting of seed, skins and stem (see **Fig. 2.3**).

As a result, many studies attempting these handling or re-utilization methods for cranberry pomace are available as shown in **Table 2.3**. From these studies, a distinction can be made between two modes of handling: traditional (or first generation) methods of handling and the biorefinery approach of valorisation, as enumerated below.

Figure 2.3. The cranberry fruit and its processing by-product—cranberry pomace [Photo courtesy of the Agricultural Research Service of the US Department of Agriculture].

2.1.2.1 First generation method

Cranberry pomace contains a variety of components, including seeds, skins as well as other uncrushed biomass. The traditional method of handling cranberry juicing waste is by landfilling and/or utilization as animal feed. Recent studies have also reported the possibility of composting this biomass; however, their natural acidity means that composting them negatively affects soil pH, nutrient availability and microbial life and activity, thus necessitating their combination with other natural or synthetic alkaline materials (e.g. chitinous fish processing waste) for their neutralizing effects (Phipps, 2014; San Miguel *et al.*, 2012). Yet this could be an expenditure which farmers are unwilling to undertake.

2.1.2.2 The Biorefinery approach

It has been revealed that this unutilized biomass is rich in both soluble and insoluble fibre and sugars, phenolic compounds, and pigments, as well as tartrate (Girard and Sinha, 2006). If a biomass containing the latter is sent to dumpsites, a sizeable environmental pollution/threat is posed. Traditionally, while cranberry wastewater is valorised for production of compounds such as food colorants, nutraceutical oil, dihydroxyacetone and tartaric acid (Blanc *et al.*, 1986), solid pomace is utilized either as animal feed (where it is largely unsuitable considering its low protein content and tartness) or, equally common, disposed of in dumpsites. However, in addition to its use as animal feed, cranberry pomace obtained from juicing industries can potentially be used for colour and tannin extraction, inks and pigments, antibacterial agents, nutraceutical products, soap and spa products, filtration and structural material, wood and leather preservatives, fuel additives as well as a substrate in solid-state fermentation (Zheng and Shetty, 1998). These perceived usefulness coupled with the environmental and socio-

economic implications of the composting route of management have spurred legislations (both in the developed and developing societies) that foster studies relating to recovery of value-added products from fruit processing residues including cranberry pomace.

Table 2.3. Select studies showing applications of cranberry pomace other than direct disposal.

Handling Class	Utilization Route	Aim/process description	Reference
First generation	Livestock feed	*Animal feed*	
	Composting	*Cranberry juicing residuals (skins, seeds, and discarded whole fruits), when applied in tandem with alkaline wastes, can ameliorate soil quality*	(San Miguel *et al.*, 2012)
		Cranberry pomace proposed as deodorizing agent (and/or co-compost) in shellfish composting	(Phipps, 2014)
Bio-refinery	Extraction	*Polyphenol extraction (with aqueous-ethanol solvent) and its stabilization with soy protein present an innovative utilization of cranberry pomace for nutraceuticals development*	(Roopchand *et al.*, 2013)
		A pilot-scale process of simultaneous juice and cranberry pomace extract was developed and optimized; extract was potent in rat and recommended for children for the delivery of antioxidant and vasorelaxant benefits	(Harrison *et al.*, 2013)
		By Soxhlet extraction, hexane was used to recover complexes of oligomeric proanthocyanidins (COPCs) from cranberry seed oilcake, with antiradical activity superior to grape seed counterpart	(Sprygin and Kushnerova, 2004)
		Red anthocyanin pigment solvent extracted from cranberry waste pulp by percolation and purified using some membrane technologies (ultrafiltration and reverse osmosis)	(Woo, von Elbe, and Amundson, 1980)
	Solid-state fermentation	*Ability of mushroom (Lentinus edodes) to release ethanol and phenolic antioxidants from cranberry pomace was investigated; production of ellagic acid is proposed*	(Vattem and Shetty, 2003)
		Cranberry pomace proved a potential substrate for producing food-grade phenolic compounds and β-glucosidase	(Zheng and Shetty, 2000)
		Cranberry pomace, when augmented	(Zheng and

	with calcium carbonate, water, and ammonium nitrate, can be used as substrate for growing sundry food-grade fungi	Shetty, 1998)
Biogas production	*Anaerobic co-digestion of animal manure and food (including cranberry) processing wastes for methane gas production was actionable*	(Jackson, Lisboa, and Lansing, 2014)
Extrusion and food development	*Extruded product based on cranberry pomace and corn starch was developed; effect of this process on the antioxidant activity of the product was assessed*	(White, Howard, and Prior, 2010a)
Film formation	*Cranberry pomace, augmented with low and high methoxyl pectin and using sugar alcohols as plasticizers, formed coloured and flavoured edible films with potential niche applications*	(Park and Zhao, 2006)

Studies indicate that a large amount of anthocyanin/pigments and other phenolic compounds present in cranberry skins and seeds are retained post-juice extraction (Skrovankova *et al.*, 2015; White, Howard, and Prior, 2010b). Moreover, when the pressing or counter-current juice extraction method is employed—that is, without using enzymatic de-pectinization through the addition of pectin-degrading enzymes—as is common in many North American cranberry juicing plants, it can result in pomace with pectin content remaining (almost) intact and available for extraction (Barrett, Somogyi, and Ramaswamy, 2005; Mantius and Peterson, 1995).

2.2 Solid–liquid extraction: a viable route for food waste valorisation

As was illustrated using cranberry juice and concentrate, much of the industrial processing of biological entities—plant and animals—into palatable and stable foods not only result in the desired product (such as juice, purees, pulp, fermented drinks, jellies, candies, ice creams, to name a few from fruit and vegetable crops), but they also inexorably result in the production of sundry side stream/by-products. These underutilized (or even unutilized) segments end up, for the most part, as waste. This has spurred a diversity of both concerns and interests on the issue: concerns from the populace, governments and the processors themselves, and interest mainly from the scientific community. Either way, there has arisen a goal of transforming these 'wastes' into products of value; waste valorisation has in fact been dubbed an attractive approach to providing an alternative to disposal and/or landfilling (Monspart-Sényi, 2012). A distinction has, however, been made between basic (composting, recycling and burning as fuel)

and advanced (green biological/chemical technologies) valorisation techniques (Luque and Clark, 2013). While the former are more robust and established, the fact that the biomass is not entirely harnessed makes the latter more appealing from the practical, economic and ecological sustainability viewpoints.

If the valorisation of food processing waste will be worth the while, it is essential that technique(s) adopted be environmentally benign and economically justifiable. Many of the common routes of utilization of fruit by-products particularly as regards the cranberry industry are shown in **Table 2.3** above. Literature presents many instances of such cascade-type techniques including the initial isolation/separation of chemicals of industrial values and even (bio)chemical/enzymatic synthesis of novel products from these compounds; microwave-assisted extraction and conversion of sugar into bioethanol are some techniques used in these routes (Luque and Clark, 2013).

Without a doubt, the extraction, particularly using novel/non-conventional technologies, of these valuable compounds is a vital starting point for their utilization, making any effort at improving this process justifiable on many fronts. Many food industry wastes have drawn attention towards their extraction, notably olive oil waste (Roselló-Soto *et al.*, 2015) and citrus peels (Ángel Siles López, Li, and Thompson, 2010; Dávila, Rosenberg, and Cardona, 2015; Pfaltzgraff *et al.*, 2013), from which compounds ranging from polyphenols, fatty acids, pigments, tocopherols, polysaccharides, phytosterols, and flavour and volatile aromatic compounds, have been recovered.

Simply put, extraction is a separation process which is based on differences in solubility (Berk, 2009). In this sense, a solvent is employed to hydrolyse and separate a solute (with higher solvent solubility) from other materials with lower solubility in the chosen solvent. When the intended solute is attached to a solid matrix, the extraction process is known as solid–liquid extraction (SLE), or leaching. Another process of extraction requires that the intended solute be originally a part of another solution, and that way, on the basis of higher solubility in another solvent, the solute is recovered; this latter process, referred to as liquid–liquid extraction or partitioning, is more common in the chemical and pharmaceutical industries, less so in food processes (Berk, 2009; Rizvi, 2010). The principles of and some improvements made to leaching are covered in this section, especially regarding its use as a food waste valorisation instrument.

2.2.1 Fundamental principles of solid–liquid extraction

Simplistically, biological materials are considered highly porous, containing a network of internal pores. Quite like many other food and chemical processes (adsorption, distillation, crystallization and dehydration), extraction is based on the transport of molecules (solute and solvent) through these pores, from one phase to another owing to a difference in chemical potential. In order words, SLE is a mass transfer process, guided by an understanding of equilibrium, material/energy balance, and mass transfer rate/kinetics (Berk, 2009).

An equilibrium relationship is developed when a solvent is in contact with a biological matrix; this relationship is described as follows:

$$K = \frac{C_e}{C_{dm}} \qquad \text{Eq. 2.1}$$

where K is the equilibrium constant, C_e is the concentration of the target compound in the solvent, and C_{dm} is the original concentration of the target compound in the dry raw material. Larger values of K are favourable in extraction processes, since higher C_e means that much of the compound that was initially in the product will dissolve in the solvent. Additionally, the magnitude of K is influenced by the solvent properties (including type, temperature, and pressure). Material and energy balance also dictates that the law of conservation of matter and energy be met at any stage of an SLE process/system. This is simply represented as:

$$mass_{in} = \text{mass}_{out} + accumulation \qquad \text{Eq. 2.2}$$

2.2.1.1 Diffusion and mass transfer

SLE of valuable biological compounds (pectin, polyphenols, and pigments) from a solid matrix has been considered as a simplistic system involving interactions between solvent, solute and solute-carrying matrix. The process (and its system), shown in **Figure 2.4**, generally involves four distinct, consecutive and recurrent steps:

(1) diffusion of solvent into the matrix's internal pores;
(2) dissolution of the target and other soluble solutes from the matrix into the solvent; and
(3) diffusion of the dissolved solute through the internal pores of the matrix to the boundary layer (i.e., between the particle surface and bulk solvent);
(4) washing off the solution from the boundary layer.

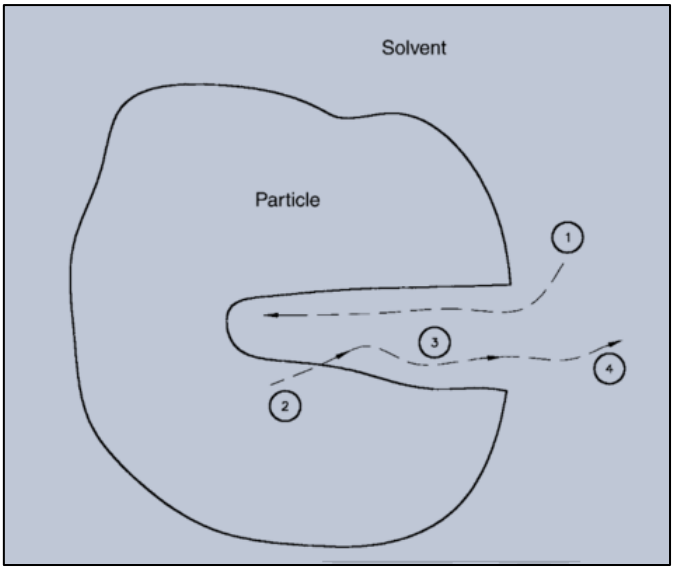


Figure 2.4. Four mass transfer events during solid-liquid extraction [Reproduced with permission from Gertenbach (2002). Copyright 2002, CRC Press].

While the second step will proceed as long as the equilibrium of the system is not attained, and with a balance of materials and energy, it carries no information about how fast the equilibrium is reached or approached. An understanding of this kinetics requires the use of mass transfer principles, and as is obvious from steps (1), (3) and (4), this principle is fundamentally diffusion-based.

Studies have shown that predominantly, step (3)—the diffusion of extract/solution through the pores—is the rate-limiting step in extraction processes (Gertenbach, 2002). This diffusion rate can be described by Fick's second law of diffusion as follows:

$$\frac{\partial C}{\partial t} = D_{eff} \times \frac{\partial^2 C}{\partial r^2} \qquad \text{Eq. 2.3}$$

where: C is the concentration of the solute in the solvent, in mol m^{-3}; t, the time in second (s); r, the radial distance coordinate from the centre of the spherical particle, in m; and D_{eff}, the effective diffusion coefficient or diffusivity of extract, in $m^2\ s^{-1}$. This equation shows that the concentration gradient between the solute and solvent provides the impetus for extraction; the rate of extraction, $\partial C/\partial t$, can be increased with a larger concentration gradient. Moreover, **Equation 2.3** shows that the diffusion rate can be ameliorated by increasing the effective diffusivity D, or by reducing the particle size r.

D values of solutes differ based on the solvent type and properties (e.g. water and temperature, respectively); it also depends on particle size as shown in **Table 2.4**. Solving the partial

differential **Eq 2.3** incorporates the particle geometry as well as the technique/equipment used for extraction (Gertenbach, 2002).

It then becomes lucid that the four parameters—solvent composition, temperature, particle size and liquid-to-solid ratio—influence the rate of an extraction process not only by affecting the equilibrium constant K but also the diffusion rate $\partial C/\partial t$. These are particularly exemplified in the industrial process by which pectin is recovered from various fruit/food processing side streams.

2.2.1.2 Production of pectin: an extraction process

The production process for pectin from various raw materials involves a series of sequential steps as outlined in **Figure 2.5**. Broadly, this would include: a pre-treatment stage, an extraction stage, and a purification or isolation stage.

a. Pretreatment

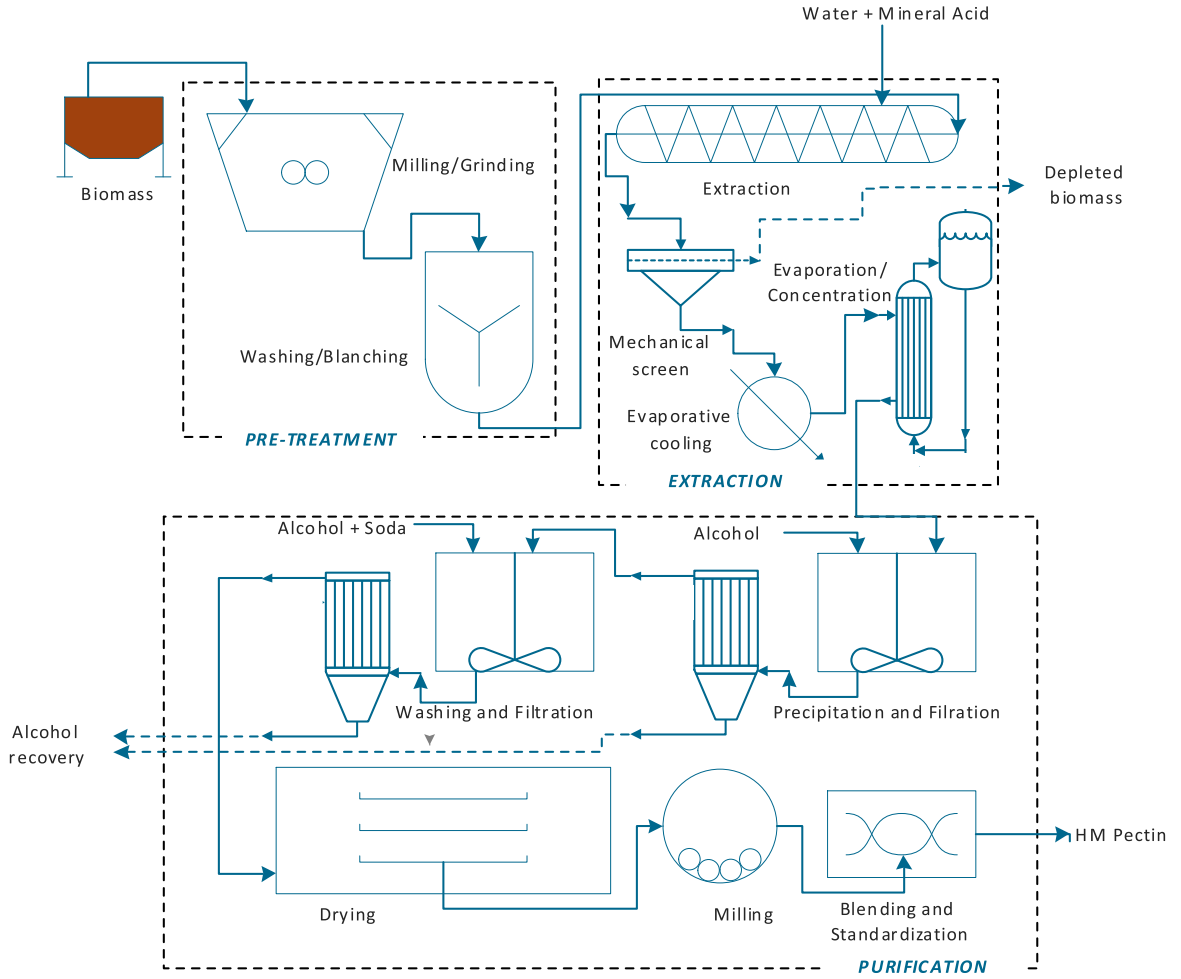
Typical pre-treatment of the choice raw material would include some or all of blanching, maceration, drying, size reduction (milling/grinding), homogenizing and sieving. Since pectin production plants are driven by inputs from other food processing industries, it means they are also faced with similar raw material supply vagaries; hence, it is often necessary for companies to store these materials during periods of plenteous supply, for use during supply off-seasons (May, 1990). However, certain raw materials (like citrus peels) contain inherent enzymes that de-esterify (and degrade) pectin and which spring into action soon after juicing of the parent fruit. It is, therefore, often the practice to either extract pectin from blanched wet fruit residue shortly after pressing the juice or to dry the materials for subsequent transportation, storage and future extraction (May, 1990). There is a theoretical inverse relation between extraction time and the square of the characteristic dimension (or particle size) of particles (Zhao *et al.*, 2013). A well-controlled size reduction is known to increase the contact surface area between solid and solvent during extraction, and it is often the norm during raw material pre-treatment in preparation for pectin extraction. Moreover, such action as grinding leads to the breakdown of cellular structure, exposing more cell wall polysaccharides thus enhancing the dissolution of pectin into the solvent.

Table 2.4. Comparison of D_{eff} of select solutes from food processing wastes.

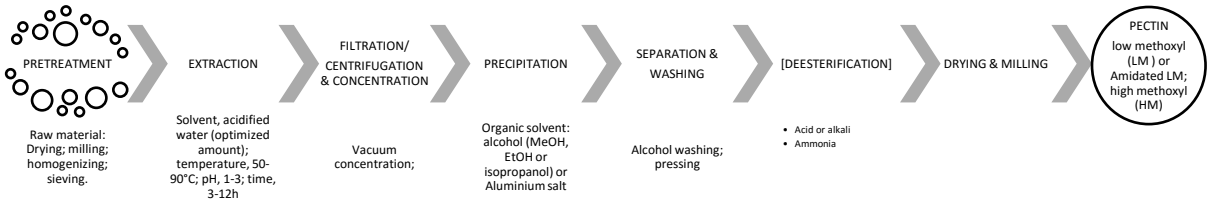
Natural product	Studied compound	Solvent	System conditions	D_{eff} ($\times 10^{-10}$ m^2 s^{-1})	Reference
Sugar beet	Sugar	Water	PEF [a] Intensity = 670 V cm^{-1}; T = 30–50 °C; r =1.5 mm.	1.30–1.74	(El-Belghiti, Rabhi, and Vorobiev, 2005)
Apple pomace	Pectin	Aqueous H_2SO_4 solution (pH 2)	T= 90 °C; r =0.2–0.81 mm	0.7–0.8	(Minkov, Minchev, and Paev, 1996)
Coffee beans	Caffeine	Water	T = 90 °C;	3.209	(Espinoza-Pérez *et al.*, 2007)
Grape pomace	Phenolics	Ethanol	T =50 °C; r = 0.5–5.5 mm	0.0105	(Pinelo, Sineiro, and Núñez, 2006)
–	Commercial quercetin	Subcritical water	T =25–140 °C; P above STP	21.80–62.60	(Srinivas, 2010)
Trachyspermum ammi	Thymol	Methanol	T = 45 °C; Microwave irradiation	0.0018–0.0032	(Gujar, Wagh, and Gaikar, 2010)

[a] pulsed electric field.

More recent pretreatments are being attempted, including brief microwave irradiation of biomaterials before conventional extraction (Kai *et al.*, 2008; Kratchanova *et al.*, 1994).



(a)



(b)

Figure 2.5. The industrial pectin production process: (a) high methoxyl pectin results; (b) de-esterification is indicated. [(a) was adapted with permission from May (1990). Copyright 1990, Elsevier].

Kratchanova *et al.* (1994) earlier reported that this practice enhanced pectin yield with an unaltered degree of esterification of resulting extract. The mechanism behind this yield and quality amelioration is the resultant inhibition of pectin-esterase activity by MW pretreatment (Kratchanova, Pavlova, and Panchev, 2004). Gentilini *et al.* (2014), equally adopted the practice (400 W microwave heating during 10 min) on Aloe vera (*Aloe barbadensis* Miller) pulp dispersed in deionized water, prior to pectin recovery. It was however reported that pectin so-obtained had an inferior molecular weight and intrinsic viscosity compared to processes without MW-heating, prompting the hypothesis that MW, coupled with acidic pH, led to the cleavage of the branched chains of RG-I and RG-II (Gentilini *et al.*, 2014). The authors further verified this pioneering observation using Fourier Transform Infrared (FT-IR) spectroscopy, with the fingerprint region (1200–800 cm^{-1}) indeed revealing a predominance of the linear homogalacturonan-backbone chain over the sidechain substituents in the RG-I and II regions.

For highly pigmented sources (such as sunflower head, and potentially cranberry), the influence of pH has been assessed for de-pigmentation. Shi et al. (1996) optimized an alkali-based de-pigmentation process using response surface methodology and reported that a slight alkaline washing of sunflower head (under conditions: pH 7.5; temperature, 16 °C; solvent to solid ratio 28 l/kg) for 25 min removed as much as 48% of the pigment with minimal (< 3.2%) pectin loss. A thirty-minute deionized water washing of Aloe vera pulp (10% w/v) at room-temperature was sufficient to remove its yellow oxidizing anthraquinone pigment (Gentilini *et al.*, 2014).

b. *Extraction*

The classical and current method of extracting pectin from pretreated biomass, known as conventional heating extraction (or CHE), is usually done in distilled water with pH (typically ranging from 1.5–3.6) lowered by the addition of a mineral acid (either monoprotic or polyprotic). Common acids used for hydrolysing native pectic polysaccharide during conventional extraction include hydrochloric acid (HCl), nitric acid (HNO_3), or to a lesser extent, sulphuric (H_2SO_4) (Jamsazzadeh Kermani *et al.*, 2015; Lopes da Silva and Rao, 2006; Seggiani *et al.*, 2009). Aqueous solutions of organic (carboxylic) acids such as citric acid ($C_6H_8O_7$) and oxalic acid ($H_2C_2O_4$) constitute some of the other solvents used for pectin extraction. Recently, for example, a patented pectin extraction process assigned by CP Kelco Aps, Denmark showed that a high quality pectin yield (degree of esterification, DE ≥ 72; and high degree of polymerization, DP, characterized by high intrinsic viscosity) can be obtained using oxalic acid and/or water-soluble oxalate as solvent (Jensen, Sørensen, and Rolin, 2013).

So an exhaustive list of hydrolysing acid might not be available. As shown in **Figure 2.5b**, this digestion process occurs at a near-boiling temperature (70–90 °C) and for a duration upward of 1 h. Clearly, process conditions need be properly monitored to ensure a reasonable quality of pectin. An understanding of the fundamental extraction principle, as well as the impact that intrinsic and extrinsic factors have on the dynamics on these principles is, therefore, important.

Extraction of pectin is also governed by mass transfer principles, whereby according to some investigations (Minkov *et al.*, 1996; Pinelo *et al.*, 2006), the outcome of the process is a function of how fast: (1) the hydrolysis of the protopectin (i.e., the *in situ* pectic polysaccharide, bound to other cell wall compounds) from the raw material occurs; (2) the resultant pectin gets solubilized; and (3) the extraction solvent attains to the state equilibrium (or saturation).

Hence, it is generally agreed that the extraction process follows two real-time events of hydrolysis and solubilisation (Cho and Hwang, 2000; Minkov *et al.*, 1996), as well as the outward diffusion of dissolved pectin into the bulk solvent. Moreover, mathematical models that integrate both the hydrolysis and the dissolution-cum-degradation events have been developed to follow a first-order chemical reaction. One of these models was used to quantify the rate of degradation of pectin, as typified by a molecular weight, and consequently intrinsic viscosity, a decrease of the extracted pectin (Cho and Hwang, 2000). Protopectin hydrolysis for a given spherical pectin-bearing particle has been shown to follow the equation:

$$\frac{dC}{dt} = -kC(1-\varepsilon) \qquad \text{Eq. 2.4}$$

where, C is the protopectin concentration, k is the rate constant of hydrolysis, ε is the porosity of the particle (and the difference, (1-ε) indicates that hydrolysis takes place with no contribution from the pores) (Minkov *et al.*, 1996). Although hydrolysis of the insoluble protopectin from other cell wall polysaccharides is integral to the successful extraction of pectin from plants, improper control of relevant factors—chiefly temperature and pH—will lead to poor quality of the resulting pectin, either in the form of extraneous co-extracts or the analyte degradation.

On the other hand, the rate-limiting step in which dissolved pectin is diffused through the pores of the solid particle to its surface has been described, with factors such as temperature and solid–liquid ratio considered important. In an extraction process temperature has been reported to influence D_{eff}, according to the Stoke–Einstein relation:

$$D_{eff} = \mathrm{k}(T/\eta) \qquad \text{Eq. 2.5}$$

where T, is the absolute temperature; η, the dynamic viscosity coefficient; and k, a constant (Loncin and Merson, 1979). However, in spite of the enhanced extraction efficiency (yield per unit time) at high operating temperatures, the somewhat heat-labile nature of pectin restricts the extent to which the temperature can be raised. This is because, at such temperatures, excessive depolymerisation, and de-esterification of the protopectin can occur, yielding extract with unattractive functional properties with limited industrial use. Another obvious fact in **Equation 2.5** is the reliance of diffusivity on viscosity; this is in line with the observable increase in the concentration of the solution as extraction progresses.

Notable factors towards obtaining quantitative and qualitative extract during pectin extraction can be broadly viewed as: technology-related issues (which includes optimal operating conditions, among them, temperature and pressure), intrinsic factors based on biomass/matrix pre-treatment condition (like particle size and moisture content) and extrinsic factors based on the solvent (like polarity, volatility, molecular weight, solid–liquid ratio, and even toxicity of the solvent used). Study of the influence of these factors on pectin extraction has previously been undertaken by El-Nawawi and Shehata (1987).

In spite of the enhanced extraction efficiency (yield per unit time) at high operating temperatures, the somewhat heat-labile nature of pectin restricts the extent to which the temperature can be raised. This is because, at such temperatures, excessive depolymerisation, and de-esterification of the protopectin can occur, yielding extract with inferior and unattractive functional properties (see **Section 2.3.4**) with limited industrial use (Lopes da Silva and Rao, 2006).

Time is another critical factor. The mass transfer process is a time-bound one, with the rate decreasing with time owing to a declining concentration gradient. More so, again with increasing concentration resulting in a more viscous extraction dispersion, the mass transfer rate is gradually impeded (Richardson, Harker, and Backhurst, 2002). The implication of this is that there is an optimal duration for the extraction of pectin beyond which, the mass transfer not only ceases, but the thermal degradation of the pectin heightens.

The ratio of solid material to extractant, known as solid–liquid ratio *S/L* (also liquid–solid *L/S*), is also considered important and should be optimised. For most investigations, *S/L* is typically represented in units of mass per volume (e.g. g/ml). Increasing the solvent percentage was shown to favour concentration gradient while decreasing the viscosity of the solution; this results in higher extraction rates, thus yields. Nonetheless, excessive L/S is detrimental for two

reasons, namely: (i) economics dictate less (or optimized) use of solvent, which would otherwise mean higher energy and wastewater disposal costs; and (ii) hydrolysis of the extracted pectin results from over-dilution of the solution (Kertesz, 1951). Kulkarni and Vijayanand (2010) extracted pectin from passion fruit peel using an aqueous hydrochloric acid (50 **wt**%) as solvent, and varied the solid to liquid ratio between 1:10, 1:15, 1:20, 1:30 and 1:40 (w/v). The authors reported that yield was significantly higher at a ratio of 1:30 than in 1:10; however, a further increase in solvent volume did not have significant effect. Hence, it was concluded that a solid/liquid ratio of 1:30 (w/v) was sufficient to give a high yield.

The pH of the extraction solvent is a most sensitive factor to note while extracting pectin. Generally, low acidic pH is necessary for the hydrolysis of the protopectin; although alkaline treatment is also useable, these have been found unsuitable because of heightened pectin degradation at neutral and high pH conditions by *β*-elimination reactions (Kravtchenko *et al.*, 1992; May, 1990). A trade-off is often made between having a better quality but low yield at less acidic pH and having a poor quality extract but with a higher yield at much lower pH; more so, this compromise is made holistically with temperature and extraction time. Typically reported values for a pH rage between 1 and 3 (Jensen et al., 2013; Lopes da Silva and Rao, 2006). Along with the acid used, the pH of the mixture can be adjusted using a minor amount of a suitable base like sodium hydroxide (NaOH).

As was mentioned previously, particle size and particle size distribution (PSD) are critical factors in pectin extraction. El-Nawawi and Shehata (1987) demonstrated that higher yields were obtained at smaller particle sizes. This is collectively due to increased surface area of contact between solid and solvent and smaller diffusion distance for the solute. But with particle size that is too fine, there is the challenge of some impedance to solvent flow through material. In addition, a small range of particle sizes is advocated, since a broad PSD means finer particles may be jammed in the crevices of adjoining coarser ones, thereby hindering solvent flow (Richardson et al., 2002). One technical factor which El-Nawawi and Shehata (1987) assessed, but which did not significantly influence extraction yield of pectin, was the agitation of the solvent. The authors posited that this was because, of the two outward diffusion processes, transport of the solute within the solid matrix is a more rate-limiting step than the diffusion of solute from matrix surface to the bulk solvent. Had the reverse been the case, the Eddy diffusion induced by agitation would have enhanced extraction yield.

c. *Filtration and purification*.

Following the aqueous extraction of extracted pectin from the now-depleted biomass, the resulting slurry is fractionated into its component (i.e., syrup-like concentrated liquid and non-pectic residue) by filtration, centrifugation, or any other similar means. An optional re-filtration/clarification may be performed to guarantee the purity of the extract, especially for food, pharmaceutical, or scientific research purposes. Pectin is insoluble in alcohols; hence, isopropanol or ethanol, but sometimes methanol, is used to precipitate it from the extract solution. This step is sometimes preceded by a concentration of the extracted solution which moderates the amount of alcohol needed for precipitation. It is often necessary to wash the precipitate with some more alcohol to exclude lingering alcohol-soluble impurities—such as sugars and phenolic compounds—followed by filtration and pressing. For most raw materials, proceeding to dry the purified precipitate so obtained yields highly methyl-esterified pectin (HM pectin). On the contrary, if before drying, the purified precipitate is subjected to a "de-esterification" step, whereby the HM pectin in alcohol dispersion is exposed either to an acidic or an alkaline condition, the product is pectin having less number of the carboxyl groups esterified by methyl, known as LM pectin. Moreover, a special kind of LM pectin, known as amidated LM pectin can be obtained if the base used for de-esterification is ammonia; this is so-called because a portion of its carboxyl groups is, in fact, amidated. Hence, in addition to the degree of methoxylation (DM), another term that defines the functionality of amidated LM pectin is the degree of amidation (DA). Function-wise, such LM pectins form gels with high degree of thermoreversibility under a broader calcium content range, more than their traditional LM pectin counterpart. This distinguishing characteristic of pectin and their functional implications are reviewed in **Section 2.3.4**.

Characterization and standardization.

The final stage in industrial pectin production involves standardization. Since biologically synthesized materials are known to exhibit a wide range of structural and functional variability, and pectin is no different, it is often the practice in pectin production to blend different batches and, in the case of HM pectin, dilute them with sucrose or dextrose to reach standard performance (Lopes da Silva and Rao, 2006). Characterization involves chemical assessment and functional (rheological) assessment of the polysaccharide.

2.2.2 Novel techniques improve solute mass transfer

From the foregoing, it is clear that mass transfer of the elements involved in SLE should be

accelerated if this process is to be improved. To that effect, a number of actionable techniques are being adopted, including, but not limited to, ultrasound-assisted extraction (UAE), subcritical water extraction (SWE), supercritical fluid extraction, pulsed-electric field extraction, and MAE. These processes not only speed up the extraction process, they also result in a greater level of selectivity, thereby improving the consistency in quality of extracted products. Other novel techniques which do not necessarily accelerate the process but which result in enhanced overall selectivity of the extraction process are however available—namely enzyme-assisted extraction (EAE).

Essentially, these SLE-assisting techniques are based on some fundamental principles of well-established technologies and differ in their manner of expediting mass transfer. A few of them as well as their principles of operation, advantages and demerits are now discussed in light of the recovery of polysaccharides and some other valuable bioactive compounds.

2.2.2.1 Enzyme-assisted extraction

a. Underlying principles and mechanisms.

With the increasing pressure on food and pharmaceutical industries to develop sustainable chemical processes (including extraction), it has become imperative to seek alternative solvent-conservative approaches such as enzyme incorporation for these processes. Enzymes are commonplace in fruit juice production processes, particularly for juice clarifying (Kashyap *et al.*, 2001; Vatai, 2013; Yadav *et al.*, 2009). Moreover, Rhein-Knudsen *et al.* (2015) reviewed EAE of seaweed hydrocolloids (carrageenan, alginate and agar) highlighting the various effects that this technique has on the physicochemical and functional properties of these marine polysaccharides.

Chiefly owing to their mode of operation, enzymes are able to catalyse reactions such as hydrolysis, with a high level of selectivity which either reduces the amount of solvent/chemical needed or increase yield for the same amount of solvent (Puri, Sharma, and Barrow, 2012), in a way that is not feasible with acid-based hydrolysis. The entangled network of polysaccharides—including cellulose, hemicellulose (like xyloglucan), and pectin—and protein in plant cell walls is such that, the cellulose/xyloglucan network is implanted in a matrix of pectin along with a protein network (Fissore *et al.*, 2009; Panouillé *et al.*, 2006). Selective cell wall component-degrading enzymes such as cellulases, hemicellulases, and proteases with minimal pectinolytic activity are often used to effect hydrolytic actions in the respective non-pectin plant cell wall components, but this requires a good knowledge both of the catalytic

action of the selected enzyme/s and of the optimum conditions for their use in EAE (Ptichkina *et al.*, 2008; Puri *et al.*, 2012).

A distinction is possible between two approaches of EAE of pectin, namely: (i) using enzymes that degrade pectin and help isolate pectin fragments e.g. galacturonic acid, and (ii) using enzymes capable of deconstructing plant cell wall and isolating pectin (Panouillé *et al.*, 2006). While the latter approach is common, examples of the former are also seen in the works by Zhao *et al.* (2015), who studied de-esterification of commercial HM pectin into LM using a synergistic application of high hydrostatic pressure (HHP) and pectin methyl esterase (PME) enzyme, and Zykwinska *et al.* (2008) who demonstrated that a direct incorporation of PME alongside cellulases and proteases into the EAE process resulted in LM pectin from an otherwise HM pectin source.

b. Operational issues for EAE

Operating EAE would typically require some level of size reduction of the pectin-bearing substrate for improved access to the cells, and like most other extraction techniques, enzymes can be selected for bespoke functionalities and for optimum process conditions (Puri *et al.*, 2012). Process conditions of particular interest in EAE processes include temperature, enzyme composition and concentration, hydrolysis time, pH, solvent–biomass ratio, and, to some extent, the moisture content of the substrate. **Table 2.5** presents several studies carried out in relation to pectin recovery using EAE. It highlights these pertinent process conditions as well as the effect of enzymes on yield and functional properties of the extracts.

c. Benefits and drawbacks of EAE

Recent studies on the use of EAE indicate a number of benefits of the method. As mentioned earlier, the need for acidic pH levels and high temperature can be eliminated using EAE. Wastewaters having these extreme conditions are typical characteristic of current industrial technology, but using these site-specific enzymes and generally low-temperature solvents can slow down equipment corrosion, while helping producer meet regulatory requirement precedent to wastewater disposal. Other advantages include: less need for rigorous pre-treatment steps (Wikiera *et al.*, 2015), which contributes to shortening the overall process time; reduced solvent consumption, and thus alcohol requirement during precipitation; and more importantly, improved structural and functional properties quality of extract, e.g. as relating to the DE and M_w of pectin (Ptichkina *et al.*, 2008; Rhein-Knudsen *et al.*, 2015; Sowbhagya and Chitra, 2010).

While these advantages of EAE exist, translation of such a promising technique beyond the laboratory scale into mainstream industrial use is currently pegged back by a number of factors. First is the cost of enzymes. Extracting pectin enzymatically in the lab scale would require relatively low concentrations. However, on the industrial scale where large volumes of raw materials are handled, their cost take another dimension making the route more expensive and less attractive to industry. For similar reasons, scale-up of EAE process can be difficult because of the uniqueness in response of different enzymes to changing environmental conditions such as temperature and nutrient availability which in turn influences pectin yield; these conditions are properly monitored in the lab, but may not be quite so in a big plant, where they can change really fast and often uncontrollably (Puri *et al.*, 2012). For these reasons, EAE of pectin is still a talk limited to academia, and the wait is on to see how the subject of scalability is resolved in the future.

2.2.2.2 Subcritical water extraction

a. Underlying principles and mechanisms

Subcritical water is liquid water at an elevated pressure which is able to attain temperatures higher than its boiling point without a change of phase. When such water is used as solvent in extraction, the process is known as subcritical water extraction—also known as pressurized hot water extraction and superheated water extraction (Zakaria and Kamal, 2015). In cases where a subcritical solvent other than water is used, the process is known as accelerated solvent extraction; hence, many studies that apply this principle for extraction within the food and environmental domains may report it in different names (Hawthorne *et al.*, 2000; Ibañez *et al.*, 2003). The elevated temperature of the water results in a number of physical advantages such as high diffusion, low viscosity, low surface tension, increased vapour pressures, and higher mass transfer rate. Furthermore, the physicochemical property of subcritical water–including dielectric and solubility–are significantly altered at such conditions (Azmir *et al.*, 2013; Zakaria and Kamal, 2015). Dielectric constant of water is around 79 at 25 °C but this reduces to 43 at 160 °C, and to 33 at temperatures around 200 °C, making it possible to extract both ionic and non-ionic compounds (Brunner, 2009; Chen *et al.*, 2015; Ueno *et al.*, 2008). **Figure 2.6** shows the typical SWE scheme as used in pectin recovery.

Table 2.5. Effects of enzymatic activities on recovered pectin and pectic oligosaccharides from plant materials.

Biomass	Enzyme class	Concentration [a] and activity	Other conditions	Effect/catalytic action on pectin	Reference
Chicory roots	Protease; cellulase; pectinase	1:10 (v/v)	8-48 h; 40 °*C* (then 100 °C post-EAE); sodium acetate buffer	~25% higher yield increase over CHE; lower MW	Panouillé *et al.* (2006)
Sundry by-products from chicory, citrus, cauliflower, endive, sugar beet	Protease, cellulose, pectin methyl esterase	1/10 (v/v)	4 h; 50 °C; 120 rpm; 1:100 and 1:50 (w/v); 0.05 M sodium acetate buffer	Healthful oligosaccharides are obtainable after EAE of pectin; HM pectin; LM pectin obtainable by addition of commercial pectin methyl esterase enzymes	Zykwinska *et al.* (2008)
Butternut *(Cucurbita moshata)*	Cellulase; hemicellulase	1:200 and 1:40 (w/w), respectively	20 h; 30 °C; 1:100 (w/v); 0.05 M sodium citrate buffer	~30% higher yield by hemicellulase; Higher DE (72.6 vs 54.2) and rheology by hemicellulase-based EAE	Fissore *et al.* (2009)
Lime peel	Cellulase; hemicellulase (xylanase, arabinoxylanse)	1/533 (v/v)	4 h; 50 °C; pH 3.5; ~1:30 (w/v); citric acid buffer.	Tweaking pH can allow EAE pectin with consistently high yield, and homogeneous/repeatable quality	Dominiak *et al.* (2014)
Lime peel	Cellulase; xylanase	0-50 *U/g*	0.5+3.5 h [b], 50; pH 4.5; 100 MPa ; 1:30 (w/v); 0.05 M citrate buffer	30 min pressure improved yield; xylanase significantly affected yield, GalA and DE	(Naghshineh, Olsen, and Georgiou, 2013)

[a] Units used for concentration varied among studies; w/w is relative to solids, while v/v relative to solvent.
[b] Combination of 30 min high-pressure processing and then 3.5 h of EAE—both at 50 °C

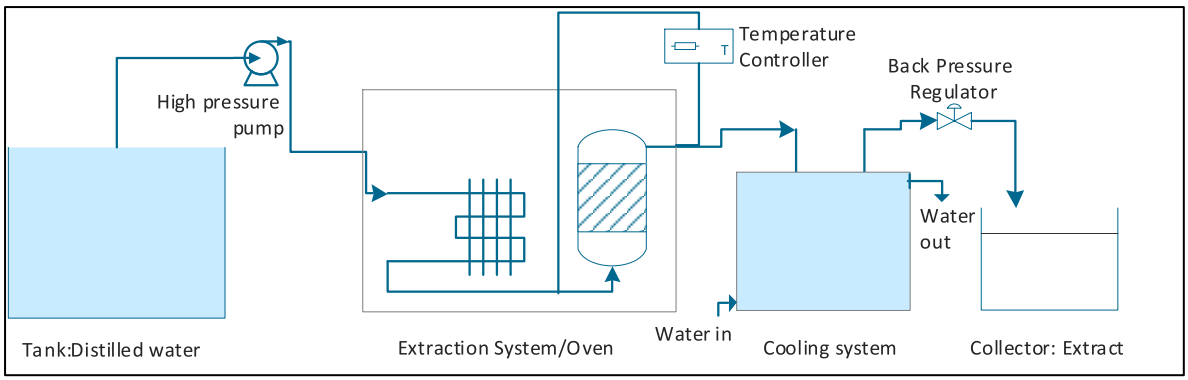


Figure 2.6. The basic scheme for the subcritical water extraction process [Reprinted from Separation and Purification Technology, 62(3), Ueno, H., Tanaka, M., Hosino, M., Sasaki, M., and Goto, M., Extraction of valuable compounds from the flavedo of Citrus junos using subcritical water, 513-516, Copyright (2008), with permission from Elsevier].

b. Operational issues for SWE

These property transformations resulting from a change in temperature condition can result in an improved extraction rate. Thus, the temperature should be given significant consideration during SWE process optimization. In pectin extraction, temperatures as high as 170 °C have been investigated. Although the primary role of pressure is to keep the water from boiling into the vapour phase (Zakaria and Kamal, 2015), this property also influences the yield of pectin as demonstrated by Chen *et al.* (2015) and some studies have investigated SWE at pressures up to 10 MPa. Furthermore, extraction time remains a key factor in SWE of pectin as an excessively long extraction period could result in degradation; hence, time should also be considered during the process optimization. The particle size of the biomass could also play a role during SWE regarding pectin yield. In addition to solvent to biomass ratio, flow rates have also been studied in continuous and semi-continuous SWE processes. Flowrates ranging from 2.1–7.0 ml min^{-1} were tested in an SWE module, and it was found that lower pressure in the vessel resulted in a haphazard (turbulent) flow regime (Ueno *et al.*, 2008). For batch operations, only *S/L ratio* are relevant in this regard (Wang *et al.*, 2014). Some of these parameters have been considered in a teeming number of studies involving SWE of environmental contaminants, pectin, and other phytochemicals, with interesting results (Chen *et al.*, 2015) (Wang *et al.*, 2014).

c. Benefits and drawbacks of SWE

With proper control of process conditions (especially temperature and pressure), high quality extracts, shorter duration and reduced solvent use, and the use of a solvent that is GRAS—

water, are some of the benefits that one can expect from SWE, thereby making the technique suited recovering food and pharmaceutical compounds. If on the other hand, poor process monitoring is adopted, excessive hydrolysis of protopectin resulting in undesirable degradation of the polymer chain, for instance in the case of pectin recovery, are encountered (Khajavi *et al.*, 2005). It is then clear that SWE demands a higher degree of monitoring and cost for its implementation.

2.2.2.3 Ultrasound-assisted extraction

a. Underlying principles and mechanisms

Sound waves with frequencies from 20 kHz (above the range audible to the human ears: 1–16 kHz) are referred to as ultrasound. These waves differ from electromagnetic waves in that, a medium (solid, liquid or gas) is required for their propagation, involving a series of expansion (which pulls molecules apart) and compression cycles (which pushes them together). For a liquid medium, the expansion cycle can create bubbles/cavities which grow and subsequently experience collapse as the negative pressure exerted exceeds the local tensile strength of the liquid. This process by which bubbles form, grow and collapse is known as "cavitation", and is the basis for UAE (Luque-García *et al.*, 2003). The cavitation process lasts about 400 μs and high temperatures and pressures, the region of 5000 °C and 1000 atm, respectively, can be witnessed (Azmir *et al.*, 2013; Luque-García and Luque de Castro, 2003).

b. Operational issues for UAE

Two modes of the *UAE system* exist, namely bath and probe units. While the former system is more generally used, two main disadvantages limit its reproducibility in extraction process: (a) lack of uniformity in ultrasound energy distribution, and (b) decline of power with time, so that the energy supplied to the bath is wasted. On the contrary, ultrasonic probes a better-off since their energy can be focused on specific sample zone yielding more efficient liquid cavitation (Luque-García and Luque de Castro, 2003; Vinatoru, 2001). For pectin extraction investigation, probe units seem to be more popular in laboratory scale studies. In implementing UAE effectively and efficiently, it is important to give proper consideration to the *plant characteristics*—including moisture content and particle size, the solvent, as well as the *hardware conditions of the ultrasound system* itself. Ultrasound system conditions will typically include *temperature*, *pressure*, *frequency,* and *sonication time*. The solvent–biomass ratio is also important since attenuation of the ultrasound intensity occurs with increasing solid particle contents (Wang and Weller, 2006).

Studies on the efficacy of UAE for pectin extraction seem to be growing with a better understanding of the ultrasound technology itself. Bagherian *et al.* (2011), in their comparison of three different extraction techniques (CHE, MAE and UAE) for pectin from grapefruit peels, found that intermittent sonication in a water bath rather than continuous sonication gave better yields. An increasing trend in extraction research has seen a synergistic use of two or more novel technologies. A case in point is the investigation by a group of researchers in China on sugar beet pulp, who not only obtained higher yield by what they called ultrasound-/microwave-assisted acid method (UMAAM), but also the pectin so obtained had much higher M_W (636.20 *vs* 295.95 kDa) in comparison to pectin obtained by traditional means (Peng *et al.*, 2015).

c. Benefits and drawbacks of UAE

UAE has a number of advantages, most notably the reduced extraction time, reduced energy consumption and a relatively lower use of a solvent (Azmir *et al.*, 2013). Further, intrinsic benefits of this extraction technique as outlined by Chemat *et al.* (2008) as related to polysaccharide extraction include: more effective mixing and micromixing, faster energy (heat) and mass transfer, reduced thermal and concentration gradients and extraction temperature, selective extraction, reduced equipment size, faster response to process extraction control, faster start-up, increased production, and elimination of process steps.

On the downside, the fact that more concentration of dispersed phase results in attenuation of the ultrasound wave means that UAE does not greatly reduce solvent requirement after all (Wang and Weller, 2006). Another potential shortcoming is that effectiveness of ultrasound in enhancing the yield and kinetics of pectin during recovery is highly biomass-specific. This means that yield varies and might not give expedited output improvement for certain materials. Further, on the instrumentation aspect, the decline in ultrasound intensity away from the vicinity of the emitter means that uniformity might not be reached for all the materials dispersed in the solvent (Wang and Weller, 2006). However, if all these factors are carefully considered, UAE can well be the technique of choice for industrial extraction of pectin.

2.2.2.4 Microwave-assisted extraction

a. Underlying principles and mechanisms

Microwave frequencies lie increasingly between the radio frequency and infrared waves. MWs are arbitrarily delimited by the frequency range 300 MHz to 300 GHz on the electromagnetic spectrum, of which a number of frequencies within this range are set aside by the International

Telecommunication Union for Industrial, Scientific, Medical and Domestic (ISM & D) applications (Raghavan, Orsat, and Meda, 2005). MWs are often used as information carriers or as energy vectors with the latter application commercially utilizing mostly frequency bands 0.915 and 2.450 GHz; most scientific and home microwave ovens in North America and Europe operate at 2.450 GHz (wavelength, 12.2 cm; energy 0.94 J/mol) (Letellier and Budzinski, 1999; Venkatesh and Raghavan, 2004). In a characteristic typical of all electromagnetic waves, MWs consist of two oscillating perpendicular fields: electric and magnetic fields. By virtue of their low photon energy, MWs are non-ionizing—that is, their energy are insufficient to break any chemical bond, nor attain to the energy of Brownian motion (**Table 2.6**) (Leonelli and Mason, 2010; Simona Mihaela Nemes, 2007). However, as many studies have now found, MW irradiation supplies sufficient internal heating for most chemical reactions, locally delivering energy at required sites, even under exothermic conditions (Leonelli and Mason, 2010).

Table 2.6. Comparison between microwave photon energy, Brownian motion energy and activation energy of major chemical bonds.

Phenomenon/quantity	**Energy (eV)**
Microwave photon	
300 MHz–300 GHz	$\sim 1.2 \times 10^{-6} – 1.2 \times 10^{-3}$
2450 MHz	$\sim 1 \times 10^{-5}$
Brownian motion	~ 0.025
Chemical bonds	
Hydrogen	~ 0.04 – 0.44
Covalent	~ 5.0
Ionic	~ 7.6

[Adapted from de la Hoz, Diaz-Ortiz and Moreno (2005) with permission of The Royal Society of Chemisty (RSC)].

Heat generation is often the desired aftermath of a microwave–matter interaction. Interactions of particular relevance involve a disorganized rotation of polar molecules of the MW-irradiated matrix (i.e., electric-field induced polarization and reorientation phenomena) which in turn creates heat. In other words, microwave heating involves the transfer of energy following two mechanisms: dipole rotation, involving the reversal of dipoles in polar molecules; and ionic conduction, involving displacement of charged ions present in the solvent (Routray and Orsat, 2012). However, heat is only generated if the material has dielectric losses, that is, if it absorbs some of the energy. Hence, the absorbed energy is obtained from the expression for loss tangent

(tan δ) (**Eq. 2.6**) and is directly proportional to the capacity of a material to be heated (Leonelli and Mason, 2010).

$$\tan \delta = \frac{\varepsilon''}{\varepsilon'} \qquad \text{Eq. 2.6}$$

where ε'' and ε' are the real (dielectric constant) and imaginary (dielectric loss factor) part of dielectric permittivity ε^* (**Eq. 2.7**), respectively:

$$\varepsilon^* = \varepsilon' + i\varepsilon'' \qquad \text{Eq. 2.7}$$

Whereas ε' expresses the ability of an irradiated molecule to become polarized by the electric field, ε'' is an indication of the efficiency of transformation of electromagnetic energy into heat (Letellier and Budzinski, 1999). It is these dielectric properties that form the basis of classifying polar and apolar solvents (**Table 2.7**), a criteria that is used during the selection of solvent–matrix systems when planning extraction processes.

Table 2.7. Dielectric constant, tangent loss and dielectric loss for commonly used solvents at 2450 MHz and room temperature.

Solvent	Dielectric constant (ε')	Tangent loss(δ)	Dielectric loss (ε'')
Water	80.4	9.889	0.123
DMSO [a]	45.0	0.825	37.125
Ethylene glycol	37.0	1.350	49.950
Methanol	24.3	0.941	22.866
Ethanol	2.4	0.040	0.096
Hexane	1.9	0.020	0.038

[a] Dimethyl sulfoxide

[Reprinted from Chemical Engineering and Processing: Process Intensification, 49(9), Leonelli, C. and Mason, T. J., Microwave and ultrasonic processing: Now a realistic option for industry, 885-900, Copyright (2010), with permission from Elsevier].

One advantage of this form of heating is the homogeneous temperature distribution in the medium; there is no temperature gradient as is the case with conventional thermal processing (Venkatesh and Raghavan, 2004). In addition to being localized, and owing to its dependencies on the dielectric and magnetic properties of the treated material, MW irradiation can be considered selective and volumetric (Leonelli and Mason, 2010). Other strong points of MW heating over the conventional heating have been said to include: rapid energy transfer, faster throughputs, fast on and off switching, compactness of equipment, clean environment at the point of use, superior moisture levelling, among others (Leonelli and Mason, 2010).

Reaction activating capacity of MW heating has over the years been adopted by a number of industries, including the chemical and food industries. Adoptions in the food industries are offshoots of many rigorous research efforts in the area of cooking, drying, baking, defrosting/tempering, lyophilisation, blanching/sterilization/pasteurization, puffing and dry-frying, and increasingly, compound and oil extraction and synthesis (Chandrasekaran *et al.*, 2012; Leonelli and Mason, 2010; Raghavan *et al.*, 2005; Veggi *et al.*, 2013; Venkatesh and Raghavan, 2004; Voss, 1974). Similar uses are found in other industries, notably rubber vulcanization in the rubber industry, timber manufacturing and efficient wood paint drying in the wood industry, sundry waste processing operations, and combined use with conventional air drying for drying in a myriad of industries.

Some of the regulatory frameworks around the use of MW units in a number of uses discussed above border around operator safety and maximum permissible leakage of MW equipment, especially in continuous flow reactors (Leonelli and Mason, 2010; Nemes, 2007). In a distance of 5 cm from the oven, a maximum MW leakage of 5 mW cm^{-2} is considered permissible; meanwhile, a maximum specific absorption rate (SAR) of 0.4 W of incident power per kg body is the prescribed limit for humans (Nemes, 2007).

b. Operational issues for microwave-assisted extraction

In MAE processes, it also important to consider the plant characteristics including moisture content and particle size. The solvent is also an important factor. Other integral factors that are considered as related to microwaves include the choice of the microwave system, the MW power density and the operating conditions like temperature.

Instrumentation for analytical studies, such as extraction, using MW often consists of either of two systems of commercially available microwave ovens or cavities, although further classifications can be made based on other metrics. They include (Letellier and Budzinski, 1999): mono-mode (or focused) microwave ovens and multi-mode (or closed) microwave ovens.

Moreover, pressure and temperature within oven can also either be controlled or uncontrolled. Fishman *et al.* (2008) reported using a temperature- and pressure-regulated closed microwave oven for pectin extraction from sugar beet. Their data showed that requisite process conditions (T, 60 °*C*; and P, 206.84 kPa) were attained in little over a minute. Conversely, the mono-mode system requires that the irradiated sample is exposed to atmospheric pressure. Accordingly, the mono-mode system is considered safer, since improper control of pressure in the closed system

can be dangerous for both user and equipment (Leonelli, Veronesi, and Cravotto, 2013). A number of recent studies have used both of these MW systems for the extraction of pectin from biomass with varying result, but the multi-mode/oven configuration is more commonly reported.

Perhaps the most influential factor on the yield and quality of pectin is the time of exposure to the MW power. Generally speaking, when irradiation time increases, there is an initial increase in yield; however, at some threshold/transition time, yield has been reported to reduce for some reasons. Hence, in MAE process optimisation, the duration of MW exposure should be considered. The role of solvent choice for MAE processes has been highlighted by Veggi *et al.* (2013); moreover, the question of what solvent to use for pectin extraction has long been answered. Hence, it is commonplace in pectin MAE research to focus on the optimal *S/L* instead. While MAE has been reported as significantly lowering solvent use, solid-liquid ratio remains an important factor that should be optimized in any MAE process, especially for extracts that form colloids in the solvent, as with the pectin. This is because solution can attain equilibrium/saturation level and become too viscous. Wang *et al.* (2007) extracted pectin from apple using MAE and observed a technical difficulty during the filtration of extracted pectin for systems containing solvents less than 14.49 ml per gram of biomass.

Table 2.8 presents some studies involving the application of MW to expedite the mass transfer of solutes from different biomass, as well as the effects of such practice. It is noteworthy that the effects of MAE are generally quantitatively and qualitatively positive on extract.

c. Benefits and drawbacks of MAE

Two obvious advantages of MAE are the reduced extraction time and lower solvent requirement. But certain underlying factors which are responsible for these two advantages can also be considered as merits as has been established in other microwave uses within the food industry. First, the non-conventional heating principle in MAE means there is a homogeneous temperature distribution within the medium with no temperature gradient. Also, equipment size can be considerably reduced. Qualitative and quantitative characteristics of MW-extracted pectin have shown improvements over conventional extraction techniques (Bagherian *et al.*, 2011; Fishman *et al.*, 1999). Hence, the MAE technology is a recognized green technology.

In spite of the reduced volume of acidified water used in MAE process (these lower ratios have more economic saving connotations), the mere fact that such acid solvent is used, and must be discarded, still brings about the corrosion problem and subsequent wear of equipment.

Table 2.8. Select studies employing microwave heating to phytochemical extraction.

Target solute	Plant Tissue/by-product	Optimal process conditions	Effects of MW on yield and quality	Reference
Pectin	Lime peels	630 W; 140 °C; 3.00 min; 25 ml g^{-1}; acid-water	High M_W (310–515 kDa); Mw, [η], radius of gyration (R_g) decreased with heating time.	Fishman *et al.* (2006)
	Apple pomace	499.4 W; 20.80 min; 14.5 ml g^{-1}; acid-water	MAE significantly lowered extraction time	Wang *et al.* (2007)
	Sugar beet pulp	1200 W; 60 °C; 3.00 min; 25 ml g^{-1}; acid-water	High M_W (~1.2 million Da) and moderate-viscosity (~4.3 dL/g) pectin recovered	Fishman *et al.* (2008)
	Mango peels	413 W; 2.23 min; 18 ml g^{-1}; acid-water	All factors considered influenced yield significantly.	Maran *et al.* (2015)
Polyphenols and caffeine	Green tea (*Thea sinensis*)	700 W; 85–90 °C; 4 min; 14.5 ml g^{-1}; aq. EtOH, MeOH or acetone	Superior yield of polyphenols and caffeine from MAE than for three other techniques	Pan, Niu, and Liu (2003)
Polyphenols	Apple pomace	650.4 W; 53.47 seconds; 22.9 ml g^{-1}; 62.1% (aq. EtOH)	Higher polyphenol yield than reflux extraction and UAE methods	Bai *et al.* (2010)
Phenolic acids and flavonoids	Tomato peels	200 W; 180 °C *; 20 min; 22.2 ml g^{-1}; water (0% EtOH)	Despite high temperature, authors submitted that developed MAE method produced functional extracts	Pinela *et al.* (2016)
Limonoid	Neem tree (*Azadirachta indica* A. Juss)	150 W; 20 min (1:1 on-off); ~30 ml g^{-1}; anhydrous MeOH	MAE results in enhanced extraction of limonoids; solvent properties influence solute selectivity by the process.	Dai *et al.* (2001)
Natural dye	Pomegranate	330 W; *Time* = 90 s; pH 3.5; ~33.8 ml g^{-1}; water	RSM and ANN ** proved sufficient for optimizing the MAE of dye process; dramatic time reduction and yield increase observed vis-à-vis aqueous extraction	Sinha, Saha, and Datta (2012)

* closed vessel system; ** response surface methodology and artificial neural network

2.3 Pectin

It should be reiterated that diverse components, serving different physiological and structural purposes, make up plant cell walls. In fruits, for example, water, cellulose, hemicellulose and pectin, make up the complex polysaccharide matrix that largely constitute the system. Other components are structural glycoproteins, phenolic esters, minerals and enzymes (Sila *et al.*, 2009). The exact interaction between these components remains an elusive theme, and a lot of research interest and effort are seen in that regard. When fruits and vegetables are processed, their functional properties—including rheological and textural properties, and ease of juice extraction—are altered, and this alteration can, in turn, be attributed to some underlying changes that occur in the cell-wall biopolymers, including not only cellulose and hemicellulose but particularly pectin. This section, therefore, briefly reviews the biochemistry, availability and broad uses of pectin.

2.3.1 Chemical and structural properties

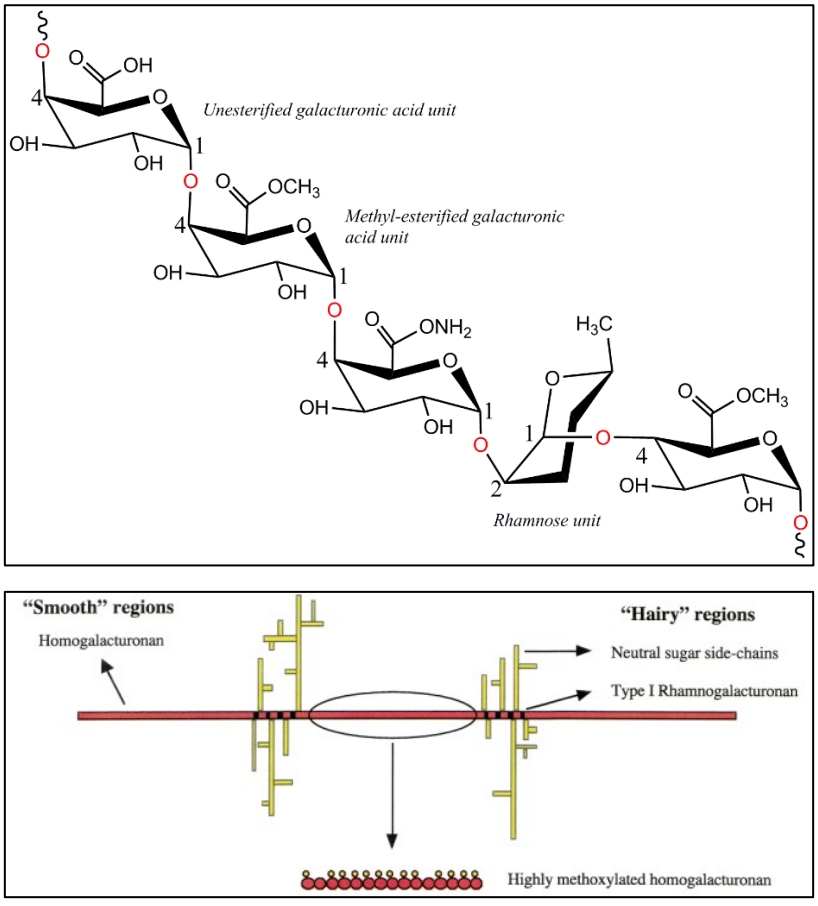


Figure 2.7. Schematic representations of pectin structure, showing GalA and Rha interruption [top] and emphasized version showing the three main structural classes [bottom; Reprinted with permission from Ralet and Thibault (2002). Copyright 2002, American Chemical Society].

Much of pectin's relevance can be traced to one or more of its structural features, including molar mass, neutral sugar content, proportion of smooth and hairy regions, ferulic acid substitution, amount of methoxyl and acetyl esters, and the distribution of the ester groups on the polymer (Sila *et al.*, 2009). Pectin is a group of the structurally complex hetero-polysaccharide polymers which contains mainly D-galactpyranosyluronic acid (GalA) as the main monomeric units linked by α-1, 4-galacturonosyl linkages (**Fig. 2.7**). The natural link is often interrupted by a variable number of L-rhamnose (Rha) units causing a discontinuity in the linear conformation of the poly(GalA) chain, also represented in the figure. These symmetry-breaking rhamnose molecules are further characterized by side chains consisting mainly of neutral sugars (**Fig. 2.7)**.The three structural classes (i.e., triad) of pectic polysaccharides, that have been isolated and characterized from primary plant cell walls till date, have been reviewed by Ridley *et al.* (2001), namely: homogalacturonan (HG), rhamnogalacturonan-I (RG-I), and substituted galacturonans (SG or RG-II) (**Fig. 2.7 [bottom]**). Some regions known as xylogalacturonan (XG) are also observable in some pectin sources.

Certain terminologies and/or protocols are used when studying or describing pectin structure; these include degree of methoxylation (DM or DE), degree of amidation (DA), degree of blockiness (DB), M_w, M_n, and degree of acetylation (DAc), as enumerated below.

The degree of methoxylation, also known as the degree of methyl esterification, or simply DM or DE. *In vivo*, pectin in plant cell wall is, to a degree, esterified with methanol, that is, hydrogen atom in some of the carboxyl (-COOH) groups along the galacturonic acid chain is replaced by a methanol (CH_3). DM is thus the percentage of the ratio of the number of moles of methanol in a pectin sample to the number of mole of galacturonic acid. Based on DE, pectins are classified into low methoxyl (LM) and high methoxyl (HM). Certain legislations, notably Codex Alimentarius, dictate that, on a theoretical scale of 0–100%, only pectins with greater than 50% DM be considered high methoxyl pectin; otherwise, it is low methoxyl pectin.

The degree of acetylation, or DAc. Certain pectins, notably those from sugar beet and potato, are acetylated on their secondary hydroxyl groups (Michel *et al.*, 1985). The presence of acetyl groups, among other reasons, has been attributed to the poor gelling ability of such pectins, where gelling is hampered through steric hindrance of adjacent chains (Lopes da Silva and Rao, 2006). In pectin extracted from sugar beet pulp, Fishman *et al.* (2008) found DA as high as 67.6%. Conversely, the less pronounced content (1%) of acetyl groups in apple and citrus pectin makes their gelling properties less influenced (Lopes da Silva and Rao, 2006).

The weight-average molecular weight (M_w) and the number-average (M_n) molecular weight. The molecular weight of pectin is another important parameter in describing pectin. Pectins, like some other biopolymers, are characterized by a high degree of polydispersity; this has meant that pectin ranging from a few tens of thousands to several hundreds of thousands Daltons (Da) have been reported from different plant source, but values between 50 000 Da and 150 000 Da are not uncommon for extracted pectin. Since D-galacturonic acid is the monomeric unit of pectin, it is important, any action that can depolymerize the pectin chain will invariable result in a decrease in M_w. So in addition to the source, other factors that can affect the M_w of pectin are the processing conditions (temperature, high shear rate, pH) as well as the actions pectin lyase enzymes. An alkaline processing condition is also believed to cause chain degradation through a β-elimination reaction (May, 1990).

*Neutral sugar profile, NS***.** As discussed earlier, intermittent interception of GalA chain creates an avenue for much of the branching that pectins exhibit. Neutral sugars such as arabinose, galactose, xylose, glucose, mannose have been identified both in isolated and *in situ* pectin from different sources (Miceli-Garcia, 2014). The content of individual NS is subject to the conditions under which pectin isolation are carried out, and a lower NS content invariably translates to a higher percentage GalA content.

*The degree of branching, DB***.** This quantity is estimated as the ratio of the respective percentage molar concentrations of galacturonic acid and rhamnose in pectin (i.e., GalA/Rha) (Yuliarti, 2011).

2.3.2 Biosynthesis

The complexity of the carbohydrate structures found in plant cell walls require a somewhat convoluted biochemical machinery for biosynthesis and construction (Caffall and Mohnen, 2009; Sila *et al.*, 2009). Since an understanding of how these cell wall polysaccharides are made is important toward modifying or regulating their production within the plant, significant effort has gone into identifying the underlying gene-encoding proteins that effect plant polysaccharide biosynthesis. However, much speculation still characterizes the biosynthetic assembly process of pectin; this subject is poorly understood, probably because of the structural complexity of pectin which informs a myriad of physicochemical properties that are important for its various biological or industrial roles (Ridley *et al.*, 2001; Sila *et al.*, 2009).

The question of how many enzymes are involved in pectin biosynthesis can also be imagined from the complexity of the pectic polysaccharide. One functional genomics and mutant study

indicated that the biosynthetic enzymes required include glycosyltransferases (GTs) and decorating enzymes like methyltransferases, acetyl transferases and feruloyltransferases (Sila *et al.*, 2009). Furthermore, of the acknowledged 412 GTs identified in the genome sequence of the model Arabidopsis, at least 53 were predicted as integral to pectin synthesis (Caffall and Mohnen, 2009; Ridley *et al.*, 2001). To further emphasize the complexity of this process, only two genes of these over 53 putative pectin synthesis GTs have been identified until date, none of which was purified using traditional biochemical techniques (Ridley *et al.*, 2001).

Synthesis of HG and RG-I-like epitopes possibly start, and continues, in the cis- and medial-compartment of the Golgi apparatus, respectively (Zhang and Staehelin, 1992); furthermore, esterification of HG is said to occur in the medial and trans Golgi, while branching of pectin happens in the trans-Golgi cisternae. The product of the pectin synthesis may occur as the Golgi vesicles are transported to the plasma membrane with the synthesized polysaccharide deposited into extracellular space between the cell walls (Ridley *et al.*, 2001). The deposited polymer is mostly in its highly methyl esterified form (i.e., HM pectin); however, un-esterified forms have also been found in the plasma membrane-cell wall interface (Knox *et al.*, 1990; Ridley *et al.*, 2001). It can thus be deduced from the foregoing that full understanding of the biosynthesis of pectin, just like amylose and amylopectin in starch, still misses some important details.

2.3.3 Physical properties and sources of pectin

In situ, pectin is found in the primary cell walls and middle lamella of many higher terrestrial plants as well as a few aquatic ones, and are normally associated with cellulose, hemicelluloses and lignin (Endress, 2011; Lopes da Silva and Rao, 2006). The content of pectic substances exhibit diversity across different plants, plant varieties, stages of growth, and even within plant tissues. They perform a number of physiologic and structural roles in plants and undergo a lot of structural changes during the ripening process, for example, of fruits and vegetables. The most widely harnessed sources of this polysaccharide are discarded portions (peels, rinds, pulps and pomace) of economically important crops like citrus fruits, apple and sugar beet, all of which are by-products of the food industry (Bump, 1995; Lopes da Silva and Rao, 2006; Thakur *et al.*, 1997). The average dehydrated apple pomace contains between 15 and 20 percent while citrus peel will contain around 30 to 35 percent (Lopes da Silva and Rao, 2006; Nelson *et al.*, 1977). Although, there are other sources, one limiting factor to their industrial utilization is the questionable structural properties of the resulting pectin (Willats *et al.*, 2006). Some of these other raw materials have been explored in the literature and even commercially, including: sugar beet, sunflower seed head, and papaya (May, 1990; Thakur *et al.*, 1997); but

there are not many reports on sources like grapes and cranberries, probably due to the low content of the compound (Baker and Kneeland, 1936) or the limited availability of the secondary raw material as compared to other industrially-used sources. **Table 2.9** shows these sources with their reported content of the polysaccharide.

Table 2.9. Sources of pectin of commercially considerable yield.

Source/utilized segment	Content (% db)	References
Commercial		
Citrus peel	30–35	(Lopes da Silva and Rao, 2006)
Apple pomace	15–20	(Lopes da Silva and Rao, 2006)
Sugar beet pulp (post-sugar extraction)	15–30	(McCready, 1966; Michel *et al.*, 1985)
Sunflower seed head	~10	(Miyamoto and Chang, 1992)
Non commercial		
Cranberry, onion, garlic, banana, mango, pumpkin, peach, rapeseed, papaya	0.1–28	(Alexander and Sulebele, 1973; Baker and Kneeland, 1936; Jeong *et al.*, 2014; Kalapathy and Proctor, 2001; Maran and Prakash, 2015; Pagán *et al.*, 2001)

Isolated pectin powder has a characteristically bright colour ranging from white, yellow, light grey or light brown (FAO/WHO, 2009), depending on the source or extraction conditions used. Regulations for its use—particularly in the food and pharmaceutical sectors—dictate that a minimum galacturonic acid level of 65% (as a food additive) or 74% (US Pharmacopeia grade) be present in a polysaccharide chain to qualify it as being pectin (EC, 1996; FAO/WHO, 2009; FCC, 2012).

2.3.4 Pectin functional properties and applications

The section discusses the applications of the compound, especially as defined by its behaviour in solution. Some of these myriad uses, particularly within the food industry, are reviewed.

2.3.4.1 Functional properties

Applications for pectin cuts across several industries, but it is the underlying structure that makes one pectin suitable for certain uses or others (Sila *et al.*, 2009). Moreover, like most polysaccharides, the use of pectin largely entails its behaviour in aqueous solutions, which spans three main concentration regimes—dilute, semi-dilute and concentrated (Walter, 1998). For example, the gel-forming ability and conditions of pectin, which is essentially in the concentrated regime, is dependent on its degree of methyl esterification (Lopes da Silva and Rao, 2006). It is on similar ground that various other uses, such as thickening and suspension

stabilization, have emerged over the years.

Gels

When a material's storage modulus is greater than its loss modulus ($G' > G''$), as obtained from a dynamic oscillatory shear test, it is said to be a gel; otherwise, it is a viscous solution (Brinker and Scherer, 1990). Many a times, food products rely on the gelation of polysaccharides in aqueous solutions in order to attain the proper structure and desired texture. In this regard, pectin gelation stems from some physical cross-linking between the HG backbones of neighbouring polymer chains; this three-dimensional networks are then capable of trapping water and other solutes within the model system (Willats *et al.*, 2006). The physical points of association are referred to as "junction zones", this is in contrast to the single-covalent linkages that is characteristic of chemically crosslinked networks (Lopes da Silva and Rao, 2006; Miceli-Garcia, 2014).

Many factors influence gel characteristics (gel strength); however, since gel formation follows markedly different routes for different classes of pectin, the degree of influence of these factors vary accordingly. In a broad sense, these factors are either intrinsic or extrinsic to the pectin. Intrinsic variables—including molecular weight, DE, the sequence of neutral sugars along the chain, the charge density, and the distribution of charge along the chain—are dependent on the vagary of the pectin source as well as the extraction method (Lopes da Silva and Rao, 2006). Of these structural properties, DE appears to be the most pertinent. Conversely, pH, temperature, ionic strength, pectin and solute concentration are the extrinsic variables which influence the rheology of pectin gels.

In the gelation of LM pectin, junction zones are formed in the presence of divalent metallic cations, mostly Ca^{2+}. This ion-mediated gelation, in which Ca^{2+} binds with ionized carboxyl groups neighbouring polymer chains, has been compared to an identical model known as "egg-box model" proposed for alginates (Braccini and Pérez, 2001; Thakur *et al.*, 1997). The gelation process is believed to follow two steps: (1) an initial dimerization, and (2) subsequent aggregation of the performed egg-box. In this model, the Ca^{2+} fits into the electronegative cavity in a twofold buckled ribbon structure of the GalA chains and involving two or more chains (**Fig. 2.8**). The concentration of the calcium salt greatly influences the rheological behaviour of LM pectin gels, increasing the magnitude of G'; however, beyond a threshold salt concentration, syneresis (or precipitation) of the pectin chain may occur (Garnier, Axelos, and Thibault, 1993).

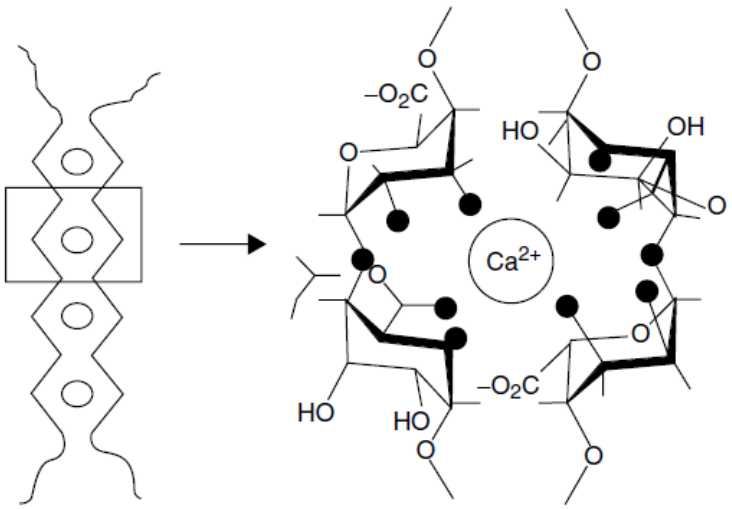


Figure 2.8. Schematic representation of the egg-box model for gelation of urinate-calcium system [Reprinted with permission from Braccini and Pérez (2001). Copyright 2001, American Chemical Society].

As for HM pectin, other kinds of molecular interactions are involved: junction zones are formed and stabilized by hydrogen bonds and hydrophobic interactions counterbalancing each other. Accordingly, ideal gel forming will include high co-solute concentration (>55%) and low pH value in the range of 2.5–3.5 (May, 1990). Typical co-solute employed in HM pectin gelation are sucrose, sorbitol, and ethylene glycol. These conditions of high sugar and high degree of acidity help to achieve the low water activity and low carboxyl group ionization, respectively, thus minimizing pectin-solvent interaction and electrostatic repulsion of methyl groups, (Lopes da Silva and Rao, 2006). It is interesting to note that gels from HM pectin are thermally irreversible upon setting (Brejnholt, 2009).

Dispersions

As mentioned earlier, the main difference between gels and dispersions (or viscous solutions) is the concentration of their solutes. As commonly found with natural polymers, the rheology (especially viscosity) of a pectin solution depends on its molecular weight, DE, and macromolecular conformation, where conformation (rigid rod, random coil, or compact sphere) is dependent upon the ionic strength, nature of counter ions, temperature, and other solution condition (Foster and Wolf, 2011; Lopes da Silva *et al.*, 1993). Furthermore, a pectin solution can either be dilute or interacting (i.e., semi-dilute).

In dilute solutions of pectin, there is a general observation of Newtonian behaviour below a concentration of about 0.5%, although the limit of this shear-rate independent behaviour

depends on DE, pH and ionic strength (Lopes da Silva and Rao, 2006). For example, **Figure 2.9** depicts the effect changing pH levels and salt concentrations have on the macromolecular conformation of pectin and other hydrocolloids.

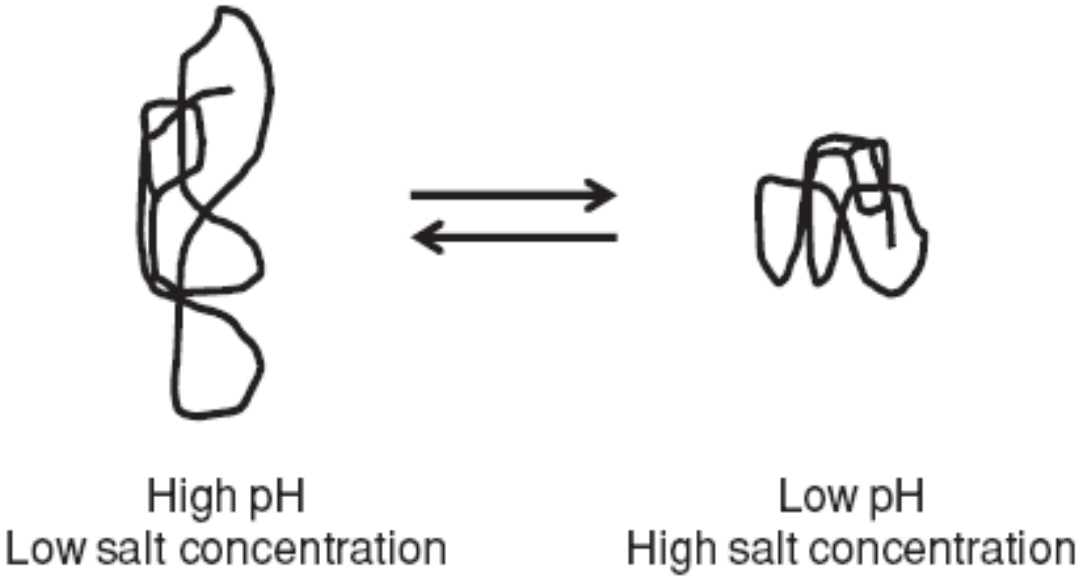


Figure 2.9. Change in the macromolecular conformation of hydrocolloids: effect of pH and salt concentration [Reproduced with permission from Foster and Wolf (2011). Copyright 2011, John Wiley & Sons, Inc.].

In addition, the intrinsic viscosity ($[\eta]$) of pectin is mainly dependent on the dimensions of the polymer chains and is the only indicator of their contribution to the viscosity of the solution at such low concentration. The radius of gyration and hydrodynamic volume are metrics used for the dimension of a polymer. **Figure 2.10** shows the three principal structures of hydrocolloids. It is clear from this figure that even for polymers having equivalent molecular weights, the variation in structure, including branching, affects the 'dimension'. Estimation of the parameter $[\eta]$ has been discussed earlier, with a unit of inverse of concentration, e.g., dl g^{-1}.

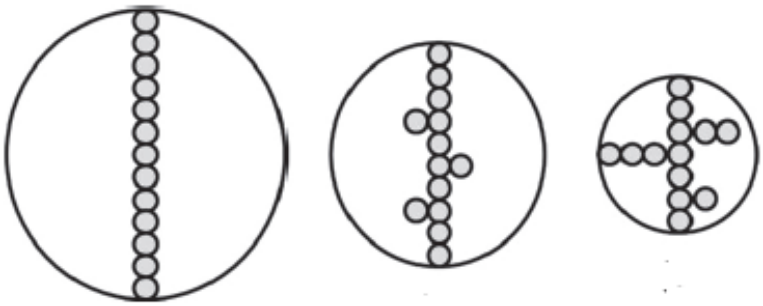


Figure 2.10. Principal structures of equivalent molecular weight-hydrocolloid polymers [Reproduced with permission from Foster and Wolf (2011). Copyright 2011, John Wiley & Sons, Inc.].

Note (1) [L-R] linear, linear-branched, and branched; and (2) their correspondingly decreasing diameter of gyration with increasing degree of branching.

A little above a nominal threshold concentration, referred to as coil over-lap concentration or critical micelle concentration (*c**), the pectin chains within the solution become increasingly entangled, leading to a higher slope of the viscosity–concentration curve (**Fig. 2.11**). The flow regime has now changed from dilute to semi-dilute (concentrated regime), and the rheology of such solution equally changes from Newtonian to non-Newtonian behaviour—specifically shear-thinning/pseudoplastic (Walter, 1998).

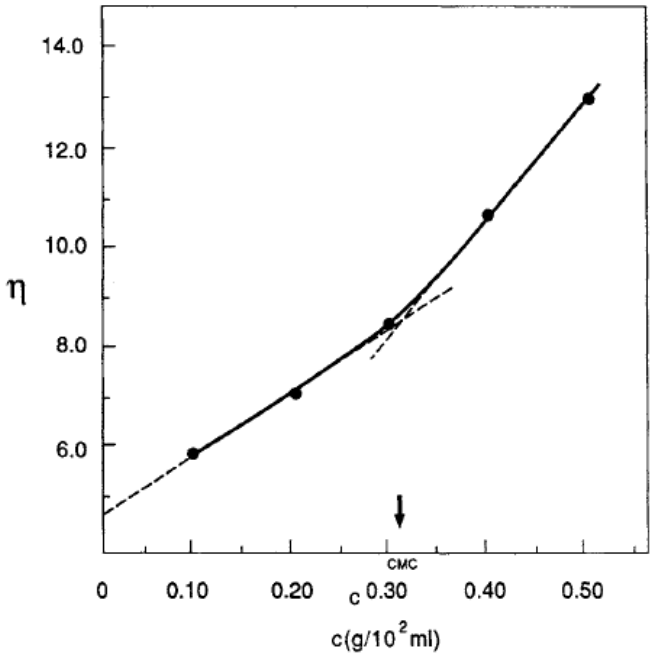


Figure 2.11. Viscosity-concentration (η vs c) profile for aqueous pectin dispersions at 26 °C highlighting change in concentration regime and the critical micelle concentration (c^*) [Reproduced with permission from Walter (1998). Copyright 1998, Academic Press].

2.3.4.2 Applications in food systems

Most commonly, pectin is used as a gelling agent, a thickener or viscosity enhancer, and a stabilizer for a number of food systems, especially beverages. These applications, as highlighted above, are primarily owing to some of their solution behaviours.

Jam, jellies, fruit preserves and marmalades. Perhaps, pectin is best known as the traditional gelling agent in jams (May, 1990). During jam formulation, especially as a function of the inherent pectin and fibre content of the fruit in use, pectin is added to improve the consistency of the food system. Indeed, the jam texture depends on the type of pectin, the sugar (co-solute) used, and the citric acid (pH) added (Rababah *et al.*, 2015). Ideally, a concentrated pectin solution (4–8%) that is to be used for jam manufacture should be prepared by adding the pectin, blended with sugar, to water in a high speed mixer (Thakur *et al.*, 1997). Moreover, LM pectin plays major roles in reduced-sugar jams since its gelation does not require sugar but calcium ion; in this case, typical concentration usage level range between 0.75–1.00 percent. Similar uses of pectin are also found in jellies and fruit preserves.

Dairy products. The dairy industry broadly uses pectin and other hydrocolloids for various purposes. In the acidic environments of acidified milk drinks below the isoelectric point (pH 4.6) of one of the milk proteins, caseins, one common problem faced is the formation of flocculated aggregates of the casein micelles (CM) (Zhuang *et al.*, 2015). This structural breakdown is attributed to the collapse of the extended conformation of κ-casein chains (Tromp et al., 2004). As a way out, Tromp *et al*. (2004), like several other researchers, in their study that in order to stabilize CM in acidified milk drink systems using 8.5% non-fat milk solids, high methoxyl pectin has to be added at a concentration around 0.3 **wt**%. Yogurts is another dairy product employing hydrocolloids. Ramaswamy and Basak (1992) investigated the influence pectin and strawberry concentrate have on the rheological properties of yogurts. They concluded that the rheology of stirred yogurt can be designed and controlled by mixing it with a suitable proportion of pectin. Pectin also acts as water binder in dairy systems.

Low fat foods and emulsions. Gums and hydrocolloids are not only used to increase viscosity and consistency of salad dressings and mayonnaise, they also help in the development of reduced-fat versions of these products for the health-conscious consumers (Ma and Boye, 2013). They help food product developers to instil the natural fat-like mouthfeel in such products while circumventing the calorific load which fat conveys. In emulsion systems, the two components (oil and water phase) have a tendency to separate into their individual phases,

but pectin is used as a stabilizer in such systems to prevent this. Similarly, pectin finds stabilizing roles in other food products like ketchup, and beverages.

Encapsulation and film-forming agent for food packaging. The gel forming capability of pectin, especially LM pectin, makes it a good candidate for encapsulating various nutraceuticals and functional food ingredients (Burnside, 2014). Antioxidants, probiotic bacteria, flavour, as well as healthful lipids and fatty acids have all been explored for encapsulation in pectin matrix (Shahidi and Han, 1993). Also, in the wake of increasing bio-based polymer research, some studies have developed active packaging films and coatings for food applications (Bierhalz *et al.*, 2012) in view of replacing the more-established synthetic ones. Pectin-based films and beads are, however, known to exhibit low hydrophobicity, as a result of which many researchers have adopted incorporation of the copolymer or using a chemically-modified variant of the compound (Liang *et al*., 2015; Piazza *et al*., 2009).

Bakery. Unsurprisingly, hydrocolloids find a niche within the bakery industry. Such uses as starch retrogradation inhibition in bread and batter (Li and Nie, 2016) and water-binding of gluten-free food (Anton and Artfield, 2008) have been reported for pectin.

Technology borrowing is a cross-industry activity, and as such, the application of pectin is no longer the food scientists' preserve. Many non-food products, and indeed industries, are now increasingly utilizing the unique colloidal properties of that pectin presents.

2.3.4.3 Applications in pharmaceutical and other systems

Regarding the safety of pectin and allied compounds, the US Food and Drug Administration's Select Committee on GRAS Substances (SCOGS) report (FDA, 1977), "*There is no evidence in the available information on pectin and pectinates, including amidated pectins, that demonstrates or suggests reasonable grounds to suspect a hazard to the public when they are used at levels that are now current or that might reasonably be expected in the future*." Based on that premise, pectin is used in some pharmaceutical preparations as excipients, thickener, stabilizer, film or coating-forming matrix, and binding agent. Sriamornsak (2003) reviewed various pectin-based carriers used for the drug delivery such as matrix tablets, gel beads, and coating.

Dietary fibre. Also, because pectin happens to be a soluble (dietary) fibre, its pharmaceutical application extends beyond only instilling functional properties; nutritional and bioactive ramifications of its application have also been reported. Since pectin is able to bind to divalent metallic cations, it acts as a natural prophylactic and has been used to remove lead and mercury

from gastrointestinal and respiratory tract and respiratory organs who have ingested heavy metal (Sriamornsak, 2003). One study reported pectin's usability in controlling haemorrhaging, because it can delay blood clotting (Joseph, 1956; Sriamornsak, 2003). More importantly, though pectin is stable to the biochemical make-up of the gastrointestinal tract, it is a ready substrate (i.e. prebiotic) for the colon microbial flora in the synthesis of healthful short-chain fatty acids (SCFAs).

Targeted drug delivery. As stated above, pectin is insoluble in the gastrointestinal fluid and resistant to enzymes present in the gastrointestinal tract, hence the justification for its use as carrier materials for colon-specific drug delivery. But being a hydrophilic polysaccharide, chemical derivatives of pectin are required when used as drug delivery systems in order to prolong the residence time of the drug (Liu *et al.*, 2003; Liu *et al.*, 2007). To this end, Semdé *et al.* (1998) investigated the suitability of pectin combined with insoluble polymers (cellulose derivatives) in developing film for drug delivery media in the presence of pectinolytic enzymes (simulating the environment of the colon, *in vitro*). They found that Eudragit RS, one of the co-polymers used, was more suitable for use with pectin as an excipient for colon-targeted drugs.

S*uspensions*. In some fronts, pectin is considered a (bio)polymeric flocculating agent. With declining reliance on inorganic flocculants such as polyacrylamide due to environmental concerns chiefly non-biodegradability, more and more applications are sought for the naturally-sourced high molecular weight polymers like polysaccharides, proteins, and glycoproteins. Perhaps the earliest reported study on the flocculating activity of pectin was at the turn of the century by Yokoi *et al.* (2002), who found and concluded pectin could flocculate organic and inorganic suspensions alike, in a way akin to the actions of the more established natural flocculants: chitin, xanthan, and poly glutamic acid. Building on this, pectin was employed in a drug suspension whereby pomelo peel pectin applied at low concentration in a complexation with ferric ions (Fe^{3+}) successfully stabilized a suspension of indomethacin (Piriyaprasarth and Sriamornsak, 2011). The authors of this study reported that type and/or presence of cations, pectin concentration and type, pH, and temperature all decide in a way the flocculating activity of pectin.

2.4 Concluding remarks

Various routes exist by which the diverse food industry by-products could be valorised. Fruit and vegetable processing, for instance, generate wastes unique in their applicability for certain

reuse (*i.e.*, pros and cons). Chiefly due to its content of sundry phytochemicals, cranberry juice processing waste—specifically pomace—has been studied using solid-liquid extraction, and if non-enzymatic juice extraction technique is utilized for juice extraction, it is expected that the polysaccharide arrangement of cranberry pomace remains intact. Thus, pectin from cranberry could be another viable target for solid-liquid extraction. Moreover, the solid-liquid extraction process has, in the past few decades, been the subject of much research and development, with the incorporation of microwave, ultrasound, and other similar devices, resulting in an amelioration of mass transfer phenomenon.

Pectin is a useful hydrocolloid within the food and other industries. Their chemical structures informs their macromolecular/hydrodynamic conformation, which are, in turn, useful for explaining the rheological behaviours of their solutions and gels. A thorough understanding of the latter serves as the basis for developing many food and other systems requiring the service of these compounds.

Evolving uses, therefore, implies increased market demand, meaning that conventional sources of pectin need to be buoyed by newer sources, in order to help suppliers keep up. Not only is new raw material exploration a plausible solution, but the industrial techniques of recovering/producing these polysaccharides also require improvement. Research into novel biomass that are rich in pectin, as well as improved extraction techniques are ongoing. Yet consideration of a new source of the polysaccharide requires that a proper prior physicochemical characterization of its pectin be undertaken. This then helps in defining one or more of the many possible uses.

Connecting text

In the preceding review, the processing routes leading up to generation of a cranberry processing waste—i.e., cranberry pomace—have been reported, with some emphasis laid on the cranberry juice/concentrate production process. Studies proposing innovative, non-disposal handling of this biomass were also reviewed and its potential as a source of pectin was mentioned. Additionally, the extraction process has been reviewed, particularly in light of novel technologies that improve the process. This was viewed in light of the recovery of pectin and other valuable compounds from food processing wastes. The various characteristics of pectin and how these decide its various applications were also reviewed. The next part of the thesis entails the use of microwave heating in the extraction of pectin from cranberry pomace. Since the process depends on multiple factors, it is necessary to optimize these parameters; hence, response surface methodology was adopted in designing the experiment used for the optimization. The result could help decide the viability of cranberry waste as a pectin source.

3. MICROWAVE-ASSISTED EXTRACTION OF PECTIN FROM CRANBERRY WASTE STREAMS: A PROCESS OPTIMIZATION STUDY

ABSTRACT

An increasing number of research studies is being devoted to understanding the functional and nutritional properties of hydrocolloids as applicable to the food and other industry. As an important first step, these compounds are sourced from materials of plant, animal or algal origin, requiring extraction for their recovery. In this work, pectin, one such compound, from cranberry was systematically extracted from cranberry pomace with minimal anthocyanin pigmentation using microwave (MW) energy. The lab-scale process, carried out in a multi-mode bench unit, was optimized using the established response-surface methodology (RSM) with a Box–Behnken experimental design. Two system-related (temperature, 40–100 °C; irradiation time, 2–10 min) and two solvent-related (pH, 1–3; liquid–biomass ratio, 20–40 ml g^{-1}) variables were considered pertinent to the optimization procedure, and their effects on cranberry pectin yield, *CBP* (mg g^{-1}) were studied. In addition to canonical analysis, determining the condition of optimum response necessitated ridge analysis; conditions at the said optimum comprised microwave irradiation of cranberry pomace at 82 °C temperature during 6.08 min and using 22.03 ml g^{-1} of acidified water solvent with pH 1.54. When these optimum conditions were experimented, pectin yield obtained matched predicted value within experimental error. Moreover, comparison with hot water bath-heating method showed potential savings on the time and solvent fronts for the optimized MW heating process, thus underscoring the efficiency and effectiveness that microwave heating brings to extraction processes. Findings from this study makes it the first attempt to recover pectic polysaccharides from cranberry using electromagnetic field energy.

Keywords: microwave; extraction; waste valorisation; pectin; canonical analysis; ridge analysis; process optimization; response surface methodology.

3.1 Introduction

Large cranberry (CB), *Vaccinium macrocarpon*, holds a place as one of North America's main berry fruits, with a couple of U.S. states and Canada's Quebec and British Columbia provinces being the largest contributors to the world production of 540 000 tonnes (FAOSTAT, 2015); its other species, known as small cranberry (*V. oxycoccus*), is more commonplace in European

countries . Although the fruit is tart hence unpalatable for direct consumption, its nutritional and health benefits have long been identified; thus, the fruit undergoes one of diverse processing routes, for the purpose of conversion into different products prior to consumption, namely sweetened dry cranberry (or SDC), and fruit juice concentrates and juice blends (requiring extraction, concentration and subsequent blending), among others. Furthermore, one of three methods are employed in CB juice extraction processes, namely: pressing, mash de-pectination, and counter-current extraction, of which the latter two are the most commonly used mainly because of their higher juice yield (Girard and Sinha, 2006). In any case, large volumes of pomace are left behind following juice extraction, constituting costs both environmental and economic, to the juice companies. However, a waste-reduction strategy does not seem feasible in such process especially giving the unattractive nutritional make-up of the by-product. In a view of solving this dilemma, several research efforts have been devoted to identifying cranberry pomace utilization routes spanning composting, use as a substrate in solid-state fermentation for phenol-producing fungi and biogas production, as well as polyphenol extraction; even novel nutraceutical and functional foods development studies have been undertaken as possible options for CB pomace valorisation (Park and Zhao, 2006; Roopchand *et al.*, 2013; Vattem and Shetty, 2003; White *et al.*, 2009; White *et al.*, 2010b; Zheng and Shetty, 2000). Most of these investigations seek to exploit the plethora of phenolic compounds still retained in the pomace after juice extraction. When non-enzymatic approaches of juice extraction are utilized (namely, pressing and counter-current extraction), it can be expected that much of the polysaccharide network within the cell walls of the pomace remain intact, especially the protopectin. This outlook then makes for the probable prospect of not only obtaining phenolic compounds from cranberry pomace, but also for obtaining pectin in a likely integrated extraction process.

As with most hydrocolloids, many food and non-food applications employ pectin (the heteropolysaccharide residing naturally in the cell wall and middle lamella of all higher plants). The compound is industrially sourced from biomass like citrus peels and apple pomace. Special kinds are also obtained from sugar beet pulp following sugar extraction, and seedless sunflower heads. An increasing body of literature explore newer biomass as potential pectin sources, such as pepper (*Capsicum annum*) (Ramos-Aguilar *et al.*, 2015) and olive oil processing residue (Rubio-Senent *et al.*, 2015); this is mainly because of the chemical uniqueness of their pectins, which could find special applications both as dietary fibres and for other functional applications. However, the first reported effort to extract cranberry pectin (CBP) was in 1936,

involving pectin precipitation from cranberry juice. The authors sought to develop a technique for direct formation of sugar jelly from cranberry by reducing its natural acid level (Baker and Kneeland, 1936). Additionally, a study involving the characterization of cranberry pomace oligosaccharides has been undertaken (Auker, 2013). It has, however, not been succinctly established how much pectin can be obtained from this biomass.

Mass transfer processes such as extraction rely on the attainment of equilibrium as well as the prior causative presence of a concentration gradient between solute and solvent and are, in effect, diffusion-driven (Berk, 2009). The diffusion coefficient of many extraction, and related, processes have been improved by the incorporation of novel techniques, such as microwave (MW), ultrasound, pulsed-electric field, and sub- and supercritical fluids, as auxiliary energy supplies (Brunner, 2009; El-Belghiti *et al.*, 2005; Luque-García and Luque de Castro, 2003; Routray and Orsat, 2012; Viganó *et al.*, 2015; Williams *et al.*, 2004; Zakaria and Kamal, 2015; Zhu *et al.*, 2016). Of these game-changing technologies, microwave-assisted extraction (MAE) has arguably received the most attention for the recovery of different bioactive compounds of plant, animal and microbial origins, probably so because of the unique nature of the MW heating event. MAE of pectin is reported to have shortened the process time significantly, along with other interrelated dividends such as better physicochemical quality of extract.

In light of the current context, this study seeks to develop a sustainable approach to recovering quantitative amount of pectin from cranberry, at a fraction of the time that would be typically required in current industrial practice. To this effect, a multi-mode microwave-assisted process is utilized for a rapid hydrolysis of the biomass, in such a way that optimum operating conditions for such yield are ascertained. For the purpose of comparison, conventional heating extraction was also attempted.

3.2 Materials and methods

3.2.1 Material

Six hundred gram packs of whole North American cranberry fruits (*V. macrocarpon*) were purchased from a local supermarket in Montreal, Quebec, Canada and were employed as raw materials for this study. The moisture content of fresh fruits was determined in triplicate as 85% (wb), using the standard oven method described in AOAC 930.04 (AOAC, 2008). Biomasses were kept frozen until use, and upon usage, frozen materials were first defrosted in a refrigerator (4 °C). All reagent used in this study were of analytical grade.

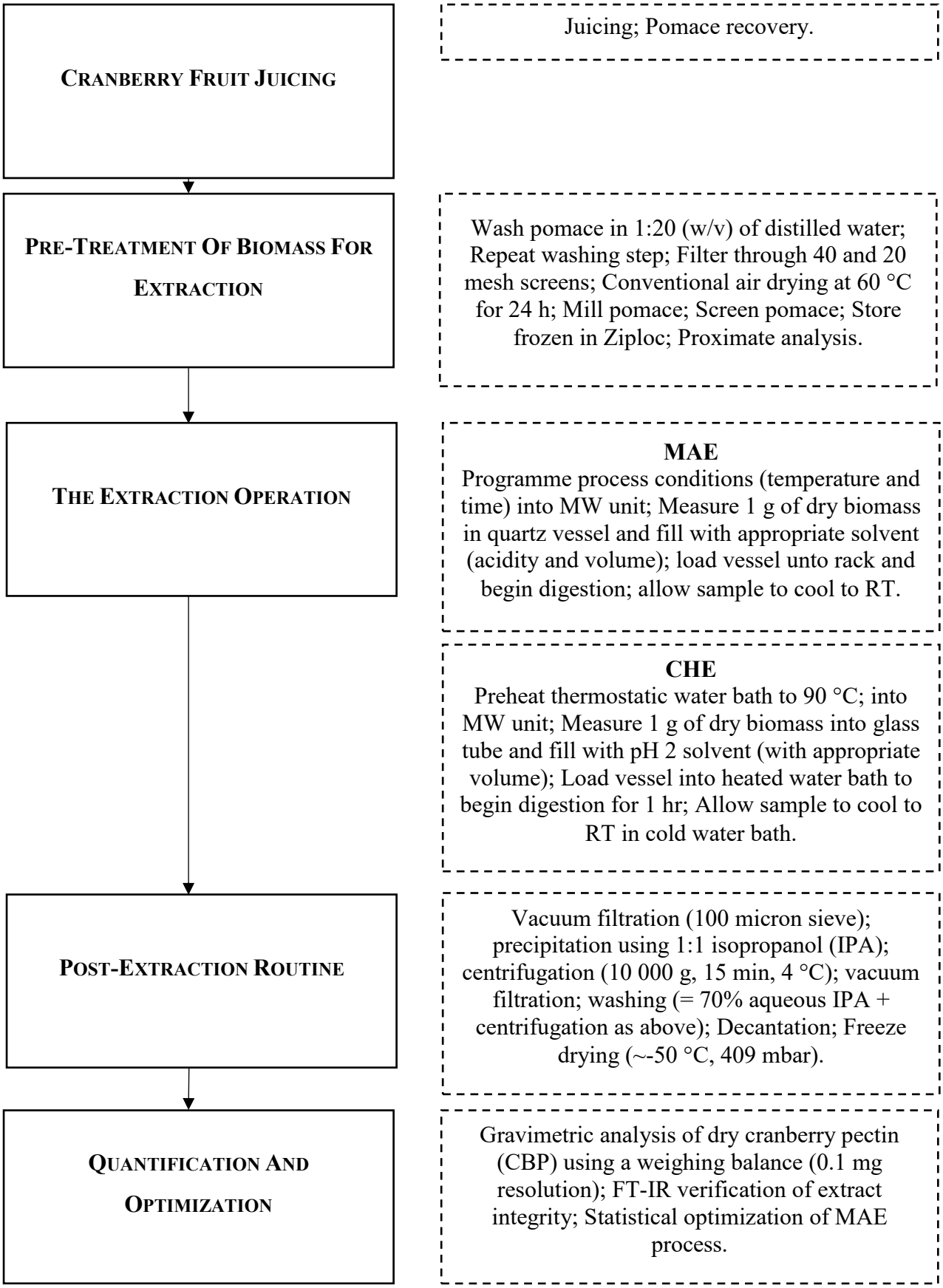


Figure 3.1. Methodology for cranberry pectin recovery.

3.2.1.1 Preliminary treatments of biological material

Pre-treatment of the cranberries followed a series of steps (**Fig. 3.1**) as adapted from Yuliarti *et al.* (2015), who studied pectin from gold kiwifruit. Washed cranberries were juiced using a Breville® fruit juicer (Model JE 900, Breville Pty. Ltd, Pointe-Claire, Quebec, Canada), with the intent of simulating operations as obtainable in a cranberry juicing plant. Notably, cranberry juice extract was very viscous; hence, juice extracts was filtered through mesh #20 under vacuum to recover solid material (including seed, skin and coarse berry pulp). This pellet was considered part of the pomace and was combined with pomace that had accumulated in the receptacle. Again, the whole pomace was used in the experiment without de-seeding as one would expect in industrial conditions. Afterwards, pooled pomace was dispersed with intermittent stirring in water at 1:20 (w/v) for 20 mins, sieved through two layers of Tyler sieve (mesh #20 and #40) and retained pomace were recovered. This washing step was repeated once and is considered an important pre-treatment particularly for highly pigmented pectin sources like cranberries and sunflower heads (Shi *et al.*, 1996), in order to obtain good quality pectin having minimal extraneous materials (e.g. pigments, glycoproteins).

Filtered juice was refrigerated at 4 °C, while washed pomace was dried to constant weight in a convective oven (T, 60 °C; *ca*. 24 h). Dried pomace was then milled using a Cuisinart Prep 9™ Food Processor (Model DLC-2009CHB, Cuisinart, Canada) and screened through mesh #4 (Tyler sieve); the cranberry pomace powders were transferred into sealed polyethylene Ziploc bags and kept frozen (-25 °C) until extraction step.

3.2.2 Extraction process

3.2.2.1 Microwave-assisted extraction (MAE)

Two techniques were employed in the isolation of pectin. Microwave-assisted extraction was compared with the traditional hot-acid extraction.

System: The extraction of pectin from cranberry pomace was completed in a multi-mode (closed) MiniWAVE microwave oven (SCP Science, Québec, Canada). The system consists of a touchscreen controller which is USB-connected with one of four possible modules. Digestion conditions in the module could be set: the power level, as well as power density, of the unit was self-regulated so as to maintain stipulated temperature, as was the pressure. The frequency and power of irradiation were 2.45 GHz and 1000 W; the duration of irradiation was user-defined, including ramp (or time taken to attain set temperature) and hold time (elapsed time while irradiating sample at set temperature). The oven houses a non-rotating digestion rack

able to hold six equidistant and radially-constructed 75 ml vessels. Vessels made of two MW-transparent materials—quartz and Teflon®—are available, but quartz was used throughout the experimentation. Six infrared (IR) sensors, located on the side walls of the oven, provide real-time temperature monitoring of each sample temperature, with their average recorded as the operating temperature. The magnetron is located below the floor in such a way that MW energy is evenly distributed across the digestion chamber for repeatability. Finally, the unit has a ventilation system, connected to the air system, for cooling the sample to room temperature, typically in ~6 min period. The MW system in operation is pictured in **Appendix A**.

MAE: Based on the Box–Behnken design (BBD) matrix created for the extraction process (**Section 3.3.2**), the parameters (i.e., independent variables) considered during this MAE process are depicted in **Table 3.1**. Solvent was HCl–acidified water with pH ranging from 1 to 3. For each run, one gram each of cranberry pomace powder was measured and introduced into one of the six quartz tubes with the corresponding volume of solvent added; for each run, the other tubes were filled with equal-molarity solvents as the sample-bearing ones. This is important to ensure a uniform dielectric property in all tubes, thereby preventing a system halt due to large temperature gradients (Soria *et al.*, 2014). Tubes were loaded into the oven, and extraction progressed under pre-set unit conditions (temperature and times) after which tube temperatures were allowed to cool to room temperature.

Table 3.1. System- and solvent-related conditions for MAE.

System-related	Temperature, *T*, °C (X_1)		Irradiation time, *Time*, min (X_2) [a]	
	40	-1	2 (2)	-1
	70	0	6 (2)	0
	100	+1	10 (5)	+1
Solvent-related	Solvent acidity, *pH*, (X_3)		Solvent–biomass ratio, LS, ml g^{-1} (X_4)	
	1	-1	20:1	-1
	2	0	30:1	0
	3	+1	40:1	+1

NB: X_1-X_4 represent the coded forms of the four factors their respective levels -1, 0, +1 shown to the right of natural variables.
[a] Values in parentheses are ramp times (in min) used to bring MW system to slated temperatures.

3.2.2.2 Conventional heating extraction (CHE)

For the purpose of comparison, pectin was also extracted using CHE, but in this case, current industrial process was simulated with the following conditions that one would expect to see in common practice: pH, 2; time, 1 h; temperature, 90 °C; and solvent-biomass ratio, 20:1, 40:1, and 60:1 ml g^{-1} (May, 1990). CHE was allowed to proceed in a quartz glass vessel with temperature regulated through a thermostatic water bath. As with MAE, accurately weighed one gram of dry biomass powder was used during this step.

To avoid any undue advantage to this method, stirring of the sample was avoided, save for initial vortex-mixing which was used to retrieve sticking pomace from the tubes (a similar practice was used for MAE); moreover, it has been shown that the eddy diffusion that agitation induces of solute does not greatly influence pectin diffusion during extraction (El-Nawawi and Shehata, 1987). Post-extraction and pectin quantification steps were similar for both extraction methods as described below.

3.2.2.3 Filtration, purification and quantification of pectin

Cooled slurries, from either extraction process, were separated using a Büchner funnel under vacuum condition (specify pressure) through a 100 micron cloth. Filtered pomace was also mechanically pressed to further recover extract, after which filtrate (extract) was precipitated in 1:1 anhydrous 2-propanol. The precipitate was separated from the alcohol by centrifugation at 4 °C with a 10 000 g relative centrifugal force (RCF) during 15 min in Sorvall™ Legend™ X1R centrifuge (Thermo Scientific, Inc.), followed by vacuum filtration to remove mother liquor.

As a washing step, coagulated pectin was then adjusted to 25 ml with 70% IPA and vortex-mixed, followed by centrifugation as described above; this way, IPA, containing non-pectin components, could be decanted and discarded (or recovered). The purified coagulum was then kept frozen at -80 °C for 6 h and lyophilized to constant weight with cap slightly ajar in a Thermo Savant ModulyoD freeze dryer (FD19555, Richmond Scientific Ltd, Lancashire, Great Britain) under the set conditions of -50°C and 0.22 mbar. The dried product is the designated cranberry pectin, CBP.

Prior to sized reduction of extracted pectin, gravimetric analysis was performed to quantify its yield (*CBP*), and this was expressed as:

$$CBP = \frac{dry\ mass\ of\ CBP\ (mg)}{dry\ mass\ of\ raw\ material\ (g)} \qquad \text{Eq. 3.1}$$

As required, samples were ground and stored frozen in lidded plastic dishes for subsequent analyses.

3.2.3 Experimental design and statistical analysis

In order to statistically optimize the parameters and response of the MAE process, knowledge obtained from a preliminary screening design did not indicate that significantly help to exclude any of the four factors considered; thus, a three-level four-factor BBD was employed—in a technique known as response surface methodology (RSM). At its core, RSM involves testing multiple variables by using special experimental designs to reduce the number of determinations needed to objectively measure several effects (Myers *et al.*, 2009). Using this mathematical and statistical process, the main, interaction and quadratic effects of the formulation ingredients on the extraction yield of CBP could be evaluated.

Table 3.2 represents the BBD matrix incorporating the four independent variables considered as having an impact on the present pectin production experiment. The variables in their natural (uncoded) forms were: (1) microwave module conditions—temperature (T, coded X_1) and irradiation time (t, coded X_2); and (2) solvent conditions—acidity (pH, coded X_3) and solvent–biomass ratio (LS, coded X_4), as symbolized in parentheses. These parameters have been shown to have a strong effect on the yield of pectin as commonly reported in the literature, as corroborated by the preliminary experiment. The three levels—low, intermediate, and high—of each factor are presented in **Table 3.1**, which in their coded forms (X_1 to X_4) are each depicted as -1, 0 and +1, respectively. These factors and their interactions resulted in 29 experimental points, namely 24 factorial points and 5 replicates at the centre point. Cranberry pectin yield (*CBP*) from each run was taken as the response (i.e., dependent variable), with a mathematical relation assumed such that: $CBP = \mathrm{f}(T, Time, pH, LS)$. Experimental data were then fitted into the following generalized second-order (quadratic) polynomial model (**Eq. 3.2**) in order to obtain regression coefficients:

$$CBP = \beta_0 + \sum_{i=1}^{4} \beta_i X_i + \sum_{i=1}^{4} \beta_{ii} X_i^2 + \sum \sum_{i=1}^{4} \beta_{ij} X_i X_j + \varepsilon \qquad \text{Eq. 3.2}$$

where: *CBP* is the response variable (yield of cranberry pectin in real values); β_0, β_i, β_{ii} and β_{ij} are the regression coefficients of the variables for intercept, linear, quadratic and interaction terms, respectively; ε is the residual of the predicted response; and X_i and X_j are the coded values of two distinct independent variables. Suitability of this model was adjudged on the basis of the magnitude of maximal, adjusted and predicted coefficient of determination (R^2) as well as the least p-values (0.05 threshold) based on analysis of variance (ANOVA) at the 95%

confidence level.

The design of experiment and data analysis were performed using the statistical software, JMP® (v 11.2.0, SAS Institute Inc.), as was the plotting of response surface and contour plots. As canonical analysis, response surfaces, and contour plots all revealed saddle point with no real maximum, ridge analysis proved necessary; and this technique was conducted using the RSREG procedure in the statistical programing software, SAS (v 9.4, SAS Institute Inc.), to compute the estimated ridge of the optimum response. The code used for performing this procedure can be found in **Appendix A.2**.

3.2.4 Verification of model

Post-optimization, the suitability of the obtained model in predicting the optimum response, *CBP*, was validated empirically. When the optimum conditions were obtained, actual MAE of cranberry pomace, the same batch as used for the experimental design, was conducted to verify the efficacy of the model at these conditions. Average value of the extracted CBP was compared to the estimated maxima from the ridge analysis.

3.3 Results and discussion

3.3.1 Experimental data, model fitting and statistical analysis

It was one aim of this study to develop a prediction model for the MAE of pectin from cranberry pomace, with high yield and attractive physicochemical, and thus functional characteristics. Presumably, the mass transfer process embodied in pectin extraction can be directly or indirectly affected by an array of extrinsic factors, including not only the system conditions, but also those pertaining to the solvent and biomass.

JMP software used for the experimental design and data analysis is capable of generating the solved second-order polynomial equation; the solution for coefficients inherently involves the method of least squares (MLS)—a multiple regression technique. Based on coded variables, the prediction model for *CBP* (mg g^{-1}) so obtained is given in **Equation 3.3**.

$$CBP = 29.46 + 17.98X_1 + 3.03X_2 - 7.45X_3 - 26.46X_4 - 6.35X_1X_2 + 1.10X_1X_3 + 2.25X_2X_3 - 12.60X_1X_4 - 2.63X_2X_4 + 11.00X_3X_4 + 1.56X_1^2 - 1.36X_2^2 + 18.41X_3^2 + 12.85X_4^2$$

Eq. 3.3

Table 3.2. Box–Benkhen design for microwave-assisted extraction process

Obs.	Pattern [a]	Natural input variables				Response variable, CBP (mg g^{-1})		Residuals[b]
		T, °C	*Time*, min	*pH*	*LS*, ml g^{-1}	Observed	Predicted	
1	0+0−	70	10	2	20	65.1	73.1	-8.0
2	−00−	40	6	2	20	27.3	39.7	-12.4
3	0000	70	6	2	30	29.1	29.5	-0.4
4	0−−0	70	2	1	30	44.8	53.2	-8.4
5	0000	70	6	2	30	29.5	29.5	+6.8
6	0+−0	70	10	1	30	45.7	54.7	-9.0
7	+0−0	100	6	1	30	77.0	73.8	+3.2
8	+0+0	100	6	3	30	70.0	61.1	+8.9
9	+−00	100	2	2	30	59.4	51.0	+8.4
10	0000	70	6	2	30	29.2	29.5	-0.3
11	00−−	70	6	1	20	137.8	105.6	+32.2
12	00+−	70	6	3	20	92.4	68.7	+23.7
13	++00	100	10	2	30	44.9	44.3	+0.6
14	−−00	40	2	2	30	18.0	2.3	+15.7
15	0−0−	70	2	2	20	45.3	61.8	-16.5
16	0+0+	70	10	2	40	29.7	14.9	+14.8
17	−+00	40	10	2	30	28.9	21.1	+7.8
18	−00+	40	6	2	40	16.4	12.0	+4.4
19	00++	70	6	3	40	21.9	37.8	-15.9
20	+00+	100	6	2	40	20.6	22.8	-2.2
21	−0+0	40	6	3	30	18.0	22.9	-4.9
22	0000	70	6	2	30	29.8	29.5	+0.3
23	0000	70	6	2	30	29.7	29.5	+13.0
24	00−+	70	6	1	40	23.3	30.7	-7.4
25	0−0+	70	2	2	40	20.4	14.1	+6.3
26	+00−	100	6	2	20	81.9	100.9	-19.0
27	0−+0	70	2	3	30	28.2	33.8	-5.6
28	−0−0	40	6	1	30	29.4	40.0	-10.6
29	0++0	70	10	3	30	38.1	44.3	-6.2

[a] Pattern presents the combination of the four factors in their coded forms signs (- and +) are magnitudes -1 and +1 respectively
[b] residuals (ε_i) are differences between observed and predicted CBP

Its equivalent in the natural variable is:

$$CBP = 227.548 + 1.861T + 6.320 * time - 120.022 * pH - 9.219 * LS + 0.002 * T^2 - 0.085 * time^2 + 18.408 * pH^2 + 0.128 * LS^2 - 0.053 * T * time + 0.037 * T * pH - 0.042 * T * LS + 0.563 * time * pH - 0.066 * time * LS + 1.100 * pH * LS$$

Eq. 3.4

In accordance with the design matrix, values for yield were estimated by plugging in the respective magnitudes of input variables (coded form) into the second-order model to obtain estimated values of CBP. These values as well as their residuals relative to actual observed responses are presented in the **Table 3.2**.

Table 3.3. Regression coefficients of the developed quadratic model for response (*CBP*)

Parameter	DF[a]	Parameter estimate from coded data	Standard error of CBP	*t*-value	p > |t|
β_0 (*Intercept*)	1	29.46	132.732875	3.96	0.0014
Linear					
β_1	1	17.98	1.491702	3.75	0.0022
β_2	1	3.03	10.244683	0.63	0.5384
β_3	1	-7.45	43.086239	-1.55	0.1427
β_4	1	-26.46	4.860676	-5.52	<0.0001
Quadratic					
β_{11}	1	1.56	0.007249	0.24	0.8148
β_{22}	1	-1.36	0.407767	-0.21	0.8385
β_{33}	1	18.41	6.524279	2.82	0.0136
β_{44}	1	12.85	0.065243	1.97	0.0691
R^2	0.82				
CV %	39.12				

[a] degree of freedom.

Model coefficients obtained by the MLS are summarised in **Table 3.3**. These constant parameters were examined with *t*-test, with results included in the same table. It may be seen from this test that linear terms of temperature and solvent–biomass ratio were highly significant ($p < 0.05$); the other two factors did not significantly impart the linear portion of the model. However, even though its linear term was not significant, *pH* proved to be the only significant quadratic term ($p < 0.05$); solvent–biomass ratio probably had the next most pronounced effect on this portion of the model ($p = 0.0691$). None of the interactions between the tested variables were of significance. Equally noteworthy is the observation that none of the combinations containing irradiation time was significant. These results are indicative of the fact that only the linear and/or quadratic effects of three key factors—temperature, solvent acidity, and solvent–biomass ratio—may be key to determining the quantitative recovery of CBP by MW energy.

Table 3.4 gives the analysis of variance (ANOVA) for the three components of second order polynomial model fitted to the response surface and reveals that contributions from the linear and quadratic components were ~61 and ~15%, respectively. Interaction effects contribute the

remaining portion making up the coefficient of determination (R^2) of the model as 0.82 (i.e., 82% of the variation could be explained by the second-order model), which when adjusted (adjusted R^2) resulted in 0.64.

Table 3.4. Analysis of variance (ANOVA) of regression parameters for the response surface model.

Regression	DF	Type I Sum of Squares	R^2	Adjusted R^2	F-Value	$p > F$
Linear	4	13057	0.61	–	11.82	0.0002
Quadratic	4	3109	0.15	–	2.82	0.0662
Interaction	6	1332	0.06	–	0.80	0.5827
Total Model	14	17500	0.82	0.64	4.53	0.0039[a]

[a] significant.

From statistical analysis (**Table 3.5**), solvent–biomass ratio and temperature were the most important factors because they significantly ($p < 0.05$) affected MAE of CBP. Within the range of experimental conditions, irradiation time and solvent acidity were not significant ($p > 0.05$).

Table 3.5. Analysis of variance (ANOVA) of the factors studied for the response surface model.

Factor	DF	Sum of Squares	Mean Square	F-Value	$p > F$
Temperature *(X_1)*	5	4697	939	3.40	0.0321
Irradiation time *(X_2)*	5	330	66	0.24	0.9383
Solvent acidity *(X_3)*	5	3372	674	2.44	0.0861
Solvent–biomass ratio *(X_4)*	5	10617	2123	7.69	0.0012

3.3.2 Model adequacy checks

Actual experimental values of CBP are plotted (**Figure 3.2**) against model-calculated values, with a fairly good fit (R^2 = 0.82). Authors, Baş and Boyacı (2007), opined that the absolute average deviation (*AAD*) is a good diagnostic statistic for a model's suitability for interpolation and optimization; they used this quantity alongside R^2 in their review of several biological and chemical studies employing RSM. *AAD* was computed for the quadratic model according to the equation:

$$AAD\ (\%) = \left\{\frac{\sum_{i=1}^{29}\left(\frac{|residuals|}{measured\ CbP}\right)}{29}\right\} \times 100\% \qquad \text{Eq. 3.5}$$

where |*residuals*| are absolute values of residual between measured and calculated responses that were reported for observations (i = 1 to 29), and the statistic was found to be 23.4%. To buttress the positive indication by the coefficient of determination, some influential plots were constructed to verify that the prediction model developed is adequate (**Figure 3.3a–d**).

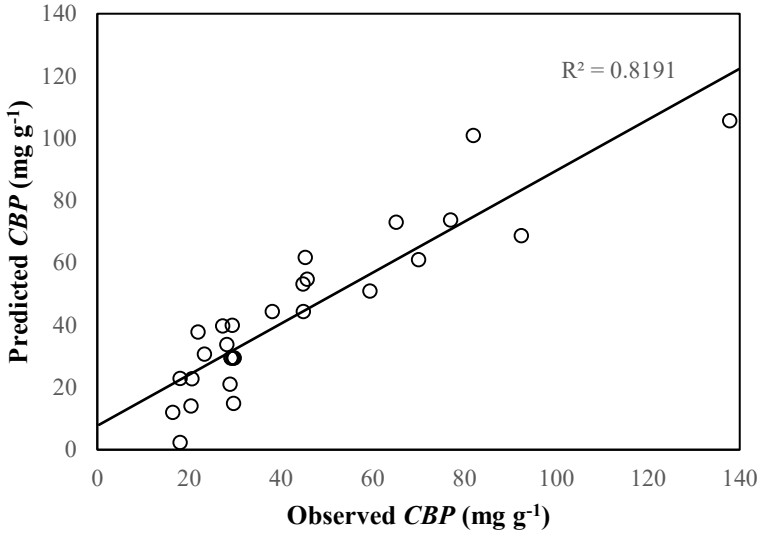
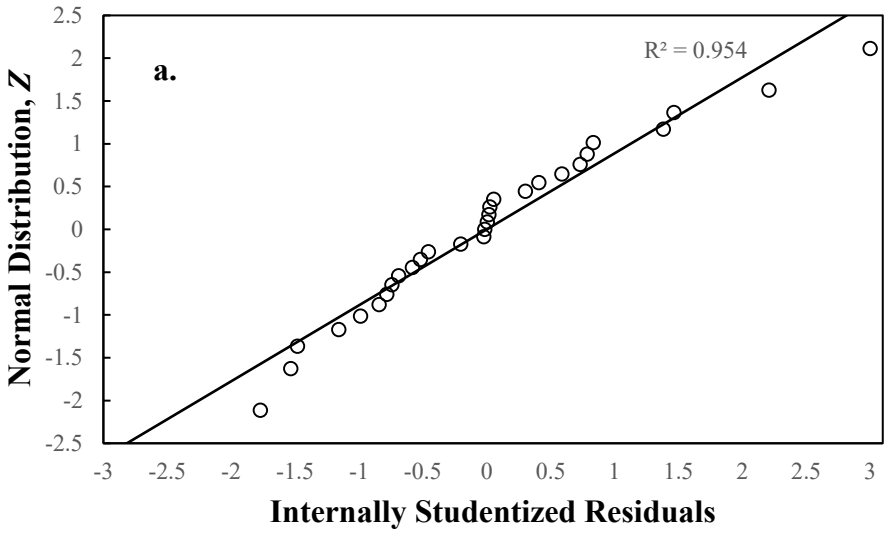
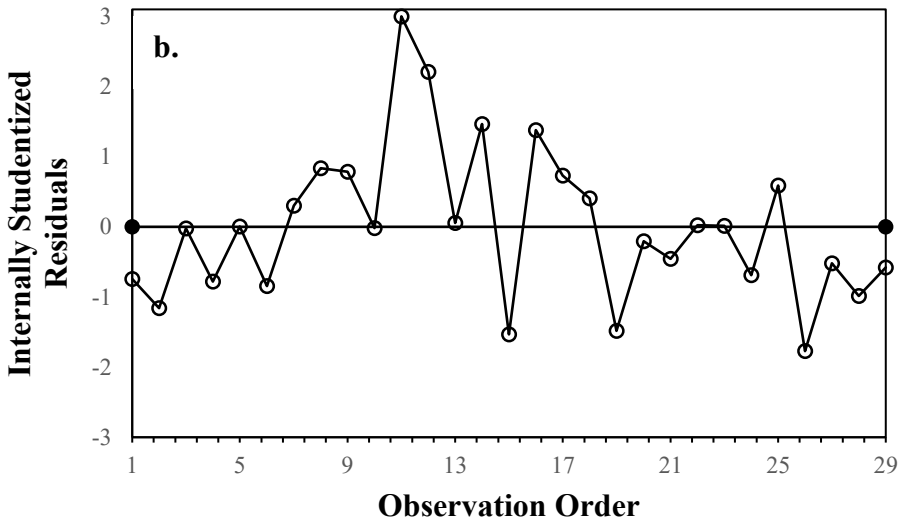
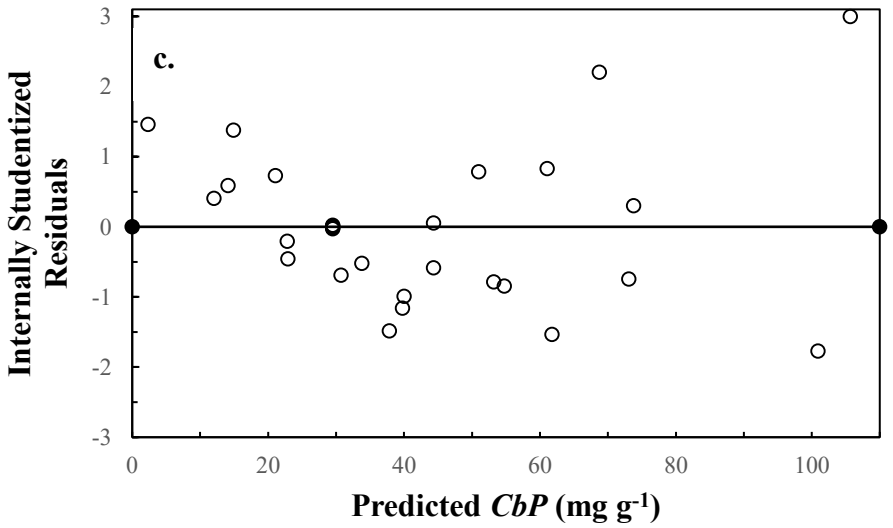


Figure 3.2. Regression plot for response *CBP*, showing predicted by actual.

Based on the recommendations by Myers *et al.* (2009), **Figures 3.3a–d** show a normal probability plot of the studentized residuals, plots of scaled residuals against predicted CBP and run order, as well as a Cook's distance (D_i) plot against residuals. None of these representations reveals any model inadequacies. The normality plot verifies the normality assumption of the residuals, with normal probabilities approaching a straight line relative to studentized residuals. Accordingly, both residual plots (**Fig. 3.3b and c**) give the general impression that the residuals scatter randomly with no obvious pattern across display, suggesting a fairly constant variance for the original CBP observations—the exception, however, was observation 11 which displayed studentized residual that approached 3. Perhaps, the most tenable explanation will be that, due to its lower alcohol–extract ratio (this point had the highest observed yield), some non-pectin materials—namely pigments, non-pectic oligosaccharides—might have survived the post-precipitation washing step, thereby amplifying the recorded weight of recovered pectin. Nevertheless, Cook's distance (D_i) plot shown in **Figure 3.3d** showed that the observation 11 might not necessarily be an influential observation, as none of the points crossed the recommended cutoff value of 1 (Myers *et al.*, 2009).


a.
R² = 0.954
Normal Distribution, Z
Internally Studentized Residuals


b.
Internally Studentized Residuals
Observation Order


c.
Internally Studentized Residuals
Predicted CbP (mg g-1)

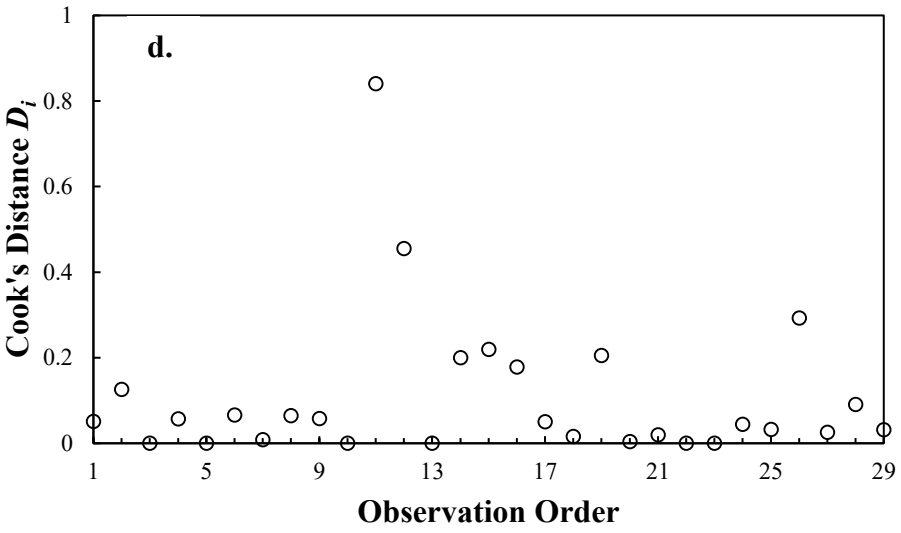
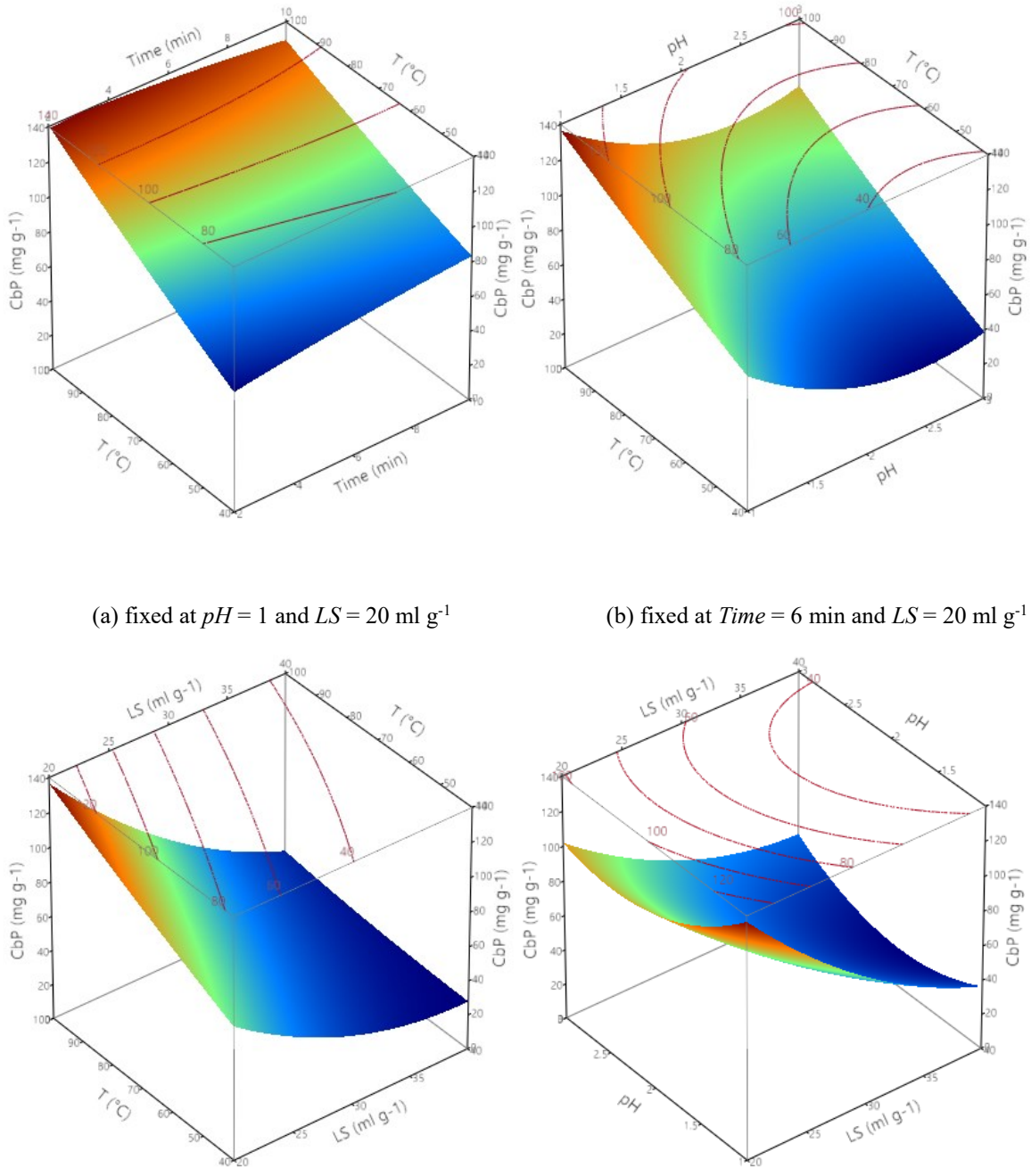


Figure 3.3. Model adequacy plots.

3.3.3 Effects of system and solvent parameters on pectin recovery

In a conventional heating extraction of pectin involving orange processing side streams, El-Nawawi and Shehata (1987) highlighted a number of pertinent factors, namely temperature, pressure, duration of hydrolysis, solvent pH and volume (i.e., *LS*), particle size and size distribution, mixing (on or off). An optimization process involving all of these potential parameters already seems too enormous a task, not to mention being uneconomical and superfluous. Indeed, a sizeable body of literature report the insignificant effect of some of them. Specifically, for example, mixing is not very paramount in polysaccharide recovery as, unlike the extraction of lysosomal compounds such as phenolic compounds, their recovery is not limited by the outward diffusion of solute from matrix surface (El-Nawawi and Shehata, 1987).



(a) fixed at pH = 1 and LS = 20 ml g^{-1}

(b) fixed at $Time$ = 6 min and LS = 20 ml g^{-1}

(c) fixed at $Time$ = 6 min and pH = 1

(d) fixed at T = 100 °C and $Time$ = 6 min

Figure 3.4. Response surface-cum-contour plots for the BBD showing effects on CBP yield of process variables.

Instead, the hydrolysis of the native "protopectin" is the more rate-limiting step here; hence, any quantity that expedites this will have a more expressive effect on the pectin yield obtained. On this premise, therefore, and coupled with some unreported preliminary laboratory experiences, four factors (temperature, hydrolysis time, solvent acidity and solvent–biomass ratio) were considered as having a foreseeable impingement on protopectin hydrolysis, and hence, pectin extraction. Although, many MW-based phytochemical extractions consider the

effects of MW power and/or power density on process responses, a mutual exclusion between temperature and MW power typically underlies this practice. In other words, if the solvent has temperature-dependent dielectric properties, the MW power is temperature-dependent or vice versa, and it is not necessary to assess both temperature and MW power effects since their magnitudes are proportional (Chandrasekaran *et al.*, 2012; Routray and Orsat, 2012). Recovered pectin from different combinations of these factors ranged from as low as 18 to 137.8 mg g^{-1} of dry cranberry pomace (i.e., 1.80–13.78 wt% db). Moreover, on the basis of collected experimental data, it was possible to visualize the interactive effects of any two of the variables (within their range of designed experiment) on the dependent variable (CBP), at fixed values of the other two factors. **Fig. 3.4** presents such 3-D response surface and contour plots which describe these interactive effects, the implication of which are enumerated as follows.

Temperature. As with any extraction process, the operation temperature of MAE is considered extremely important, especially in closed-vessel (multi-mode) microwave systems. In **Figures 3.4a–c**, one observes that temperature increase has a positive (nearly linear) relationship with CBP, within the range studied (40–100 °C). In fact, this observation is consistent for all interactions of temperature with other factors. Nevertheless, the steepness of yield increase decreased, as shown in the *T–LS* interaction plot (**Figure 3.4c**), with *LS* increase from 20–40 ml g^{-1}. This is in contrast with the report by Thirugnanasambandham, Sivakumar, and Prakash Maran (2014), who observed that an initial increase in dragon fruit peel pectin yield between 35 and 45 °C was followed by a decline in yield as temperature reached 55 °C. The difference between both studies, however, potentially lies in the duration of the process: the authors studied extraction process between 5 and 25 min which was a far call from the 2–10 min in this study. Perhaps, this quadratic propagation of yield (relative to temperature) becomes less obvious at shorter irradiation durations. Higher temperature translated to higher yield because higher temperatures result in lower surface tension, viscosity, and thus diffusivity of the solvent into the sample matrix (Soria *et al.*, 2014). This should hypothetically result in quicker and more complete hydrolysis of protopectin. Yet while an almost direct proportionality ($CBP \propto T$) was reported here for the temperature, one cannot rule out the possibility of yield decline as the upper limit of extraction time extends beyond 10 min. Conversely, **Figures 3.4a–c** seems suggestive of the fact that raising temperature beyond 100 °C while lowering the time could foster yield, just as experienced. However, practical energy cost implications of operating systems at such elevated temperatures have to be factored in, especially when scale-up and

industrial integration are targeted. Also, it must be noted that while lab-scale closed-vessel MW units are well built to bear the accompanying elevated pressure, structures with equivalent structural integrity will be required in largescale production—this, by implication, means added costs. More importantly, pectin content in cranberry pomace is finite and it cannot be said that more yield can be obtained beyond the conditions studied.

MW irradiation time. If significant quantity of native protopectin is to be hydrolysed by the traditional heat transfer from a heating element/boiling liquid bath, 10 min is certainly not sufficient, much less 2 min. But the volumetric nature of microwave heating induces a form of heating which will ideally be spatiotemporally uniform. Impliedly, given the right conditions (i.e., MW power and power density), temperatures in MW-absorbing solvents can reach astonishingly high levels in the fraction of the time. Within the range of irradiation time (2-10 min) of the cranberry samples, CBP yield exhibited a dichotomy of behaviours (**Figure 3.4a**): first, there was a subtle and somewhat linear increase in recovered pectic polysaccharide with increasing time at lower temperature (~40 °C), then as temperature approached 100 °C, a general decrease in CBP ensues at longer times. Accordingly, exposure of sample to MW irradiation beyond 6 min could actually be counter-productive to the process bottom line. In a method development study involving MAE of secoisolariciresinol diglucoside (SDG) from flaxseed (Nemes and Orsat, 2009), irradiation time was one of the two factors screened out before proceeding to an eventual central composite design optimization using MW power and alkali molarity; the 'time' factor was discarded from the screening model developed because its effect had both a negative effect and a small slope relative to SDG yield. When Fishman *et al.* (2006) attempted MAE of lime peel pectin at elevated temperature (and pressure) of about 140 °C (and 50 ± 2 psi), they found that the overall yield peaked at after 4 min; prolonging the process at such temperature–pressure condition not only resulted in lower yield, but also lower physicochemical properties of extracted polysaccharide. This 'peaking' phenomenon has been frequently observed and explained by the fact that keeping hydrolysed pectin at elevated temperature for extended duration results in a degradation, namely, depolymerisation of the chain (Prakash Maran *et al.*, 2013; Soria *et al.*, 2014); this perhaps explains the slight but ostensible drop in *CBP* around 100 °C as *time* increased from 2 to 10 min noted earlier. It can thus be concluded that when temperature is too great and solvent is too acidic, the duration should not be prolonged as this has an eventual negative effect.

Also, ignoring temperature ramp time differences used at different temperatures (noted in **section 3.2.3**) does not necessarily circumvent the effect that the same will have on the overall

MW irradiation time and the CBP yield; really, samples exposed to 100 °C were 3 min better of their 40 °C- and 60 °C-counterparts in terms of MW exposure as the temperatures rose from steady state. If that would help, one could view the process times more holistically (ramp time + hold time). In general, however, time, is only shown to have a statistically marginal influence on the herein reported extraction process.

Solvent acidity. The presence of microwave-absorbing solvents such as water, acidified water, and polar solvents are essential to the temperature increase of an MAE process. The use of acidulated water in pectin extraction stems from the fact that hydrolysis of protopectin occurs in non-neutral pH conditions. But beyond hydrolysis and dissolution, different degrees of acidity can result in different degrees of a possible re-adsorption of extracted pectin to the previously depleted plant matrix, especially during the cooling step (Soria *et al.*, 2014). Hence, pH is an important candidate to be considered during MAE optimization. **Figures 3.4b and c** encapsulate some pH effects and reveal that an initial decline in yield with increasing acidity (i.e., reducing pH from 3) is followed by a rise in yield as pH approached 1; indeed the quadratic effect of this quantity is very telling on the CBP yield. This upward-facing quadratic impact of *pH* is in contrast with the submission of Prakash Maran *et al.* (2013), who also reported a quadratic effect, but with a downward-facing function, albeit between pH 1 and 2; as pH is a very sensitive parameter, the rise in CBP yield covered by the present model (**Eq. 3.4**) from mid-pH (~2) to pH 1 might in fact be too simplistic to the point of masking another quadratic behaviour—one which the authors established in that range (i.e., pH 1–2). In other words, the higher yield might actually be between pH 1 and 2 as against what the response surface plots suggest, as was the case in MAE of pectin from apple bagasse (Wang *et al.*, 2007).

Solvent–biomass ratio. A look at response surface plots (**Figs. 3.4(c and d)**) containing solvent–biomass ratio (LS) reveals that this factor has a negative effect on CBP yield. For reason quite unclear, this occurrence was also reported by Wang *et al.* (2007), who optimized MAE of pectin from apple pomace using RSM. In their study, pectin yield appeared surprisingly improved at high solids content. The reason for this contradiction, has been tied to the higher difficulty of homogeneous heat dissipation within extraction medium at higher solvent volume (Soria *et al.*, 2014). The observation in this scheme is, however, in divergence from what is observed during recovery of smaller molecular sized compounds, as with the MAE of polyphenols and caffeine from green tea leaves (Pan *et al.*, 2003). While these (extraction of smaller compounds) align with the usual expectation of larger concentration gradients leading to higher extraction efficiencies, the polydisperse nature of pectin means that

more solvent volume will result in minimal entanglement of polymer chain, and thus, smaller molecular sized chains do not get precipitated during the post-extraction steps—instead those small enough can be lost during filtration. Whereas, using 20 ml g^{-1} *LS*, fosters the said entanglement, which explains its observed higher CBP yield. Perhaps, introducing a concentration step prior to precipitation will have increased this entanglement (May, 1990), but this was not attempted. One can, however, speculate that lower *LS* range (≤20 ml g^{-1}) will obey the direct positive relationship between solvent volume and yield and expect a peak yield around 20 ml g^{-1} as many MAE studies report. Indeed, the lower solvent–biomass ratio documented herein is industrially desirable especially in the context of environmental sustainability (Pinela *et al.*, 2016).

Polysaccharides networks are known to form gels in aqueous solutions, whose viscosities can be reduced under low pH conditions such as those herein used for extraction. However, there exists some threshold solvent volume–biomass mass ratio (*LS*) for various extraction processes involving biomass having significant polysaccharide network (or those whose target compounds are themselves polysaccharides, as in pectin). In MAE of apple pectin, authors reported the onset of the extraction/filtration difficulties at *LS* values below 14.5:1 ml g^{-1} (Wang *et al.*, 2007). Similarly, 50:1 ml g^{-1} was used in a research involving MAE of SDG from flaxseed, since lower solvent volumes exhibited viscosities that were too high to the point of inhibiting subsequent chromatography (Nemes, 2007). In the present study, *LS* below 20:1 ml g^{-1} seemed impractical for cranberry pectin extraction not only because of observed agglomeration rather than dispersal of pomace powder, but also due to a downstream filtration impediment, which could invariably skew the extract quantification.

3.3.4 Process optimization for MAE

3.3.4.1 Location and nature of the stationary point—canonical analysis

The regression models developed (**Eq. 3.3 and 3.4**) were used to establish the optimum conditions of process (independent) variables, which will result in the maximum response (*CBP*). To this end, respective first and second order partial derivatives of the model (i.e., *CBP*) with respect to the independent variables (*T*, *Time*, *pH*, i.e. *LS*) are required. This technique of determining the location and nature of the stationary point underlines the canonical analysis technique. In **Table 3.6**, the response at this stationary point is 15.0 mg g^{-1}; however, it is clear that this cannot be the maximum point since runs 11 and 12, for example, present much higher values of *CBP*. Moreover, the value of *LS* at this stationary point is seen to be slightly out of the domain of experimentation. The eigenvalues and eigenvectors are characteristics of the

response surface shapes (**Table 3.7**). Since eigenvalues are a mixture of signs, the stationary point is neither a maximum or minimum but a saddle point (as is obvious from all contour plots in **Figures 3.4a–d**).

Table 3.6. Canonical analysis of independent and response variables at stationary point.

Factor	Critical Value		Label
	Coded	Uncoded	
T	-0.27068	61.879605	Temperature (°C)
t	0.649877	8.599509	Irradiation Time (min)
pH	-0.134358	1.865642	Solvent Acidity
LS	1.021083	40.210835	Solvent–Biomass Ratio (ml g^{-1})
Predicted CBP at stationary point: 15.001477 mg g^{-1}			

Consequently, it is important to ascertain what the optimum values of the yield is using other means. This must be within the studied region of factors, since the fitted model is only good within the confines of the designed experiment (Myers *et al.*, 2009). This necessitated that a ridge analysis be carried out.

Table 3.7. Canonical analysis showing Eigenvalues and Eigenvectors.

Eigenvalues	Eigenvectors			
	T	*time*	*pH*	*LS*
22.24840	-0.157008	0.027667	0.80464	0.571959
12.24077	0.434015	0.013169	0.576343	-0.692305
2.03846	0.615655	-0.720715	-0.075196	0.309652
-5.07263	0.638709	0.692554	-0.121357	0.312558
Stationary point is a saddle point.				

3.3.4.2 Ridge analysis

Ridge analysis is a constrained optimization procedure used in RSM when the analyst finds the optimal condition of a process, as predicted by a polynomial (e.g. quadratic) model, is a saddle point (Myers *et al.*, 2009). Being akin to the method of steepest ascent in linear models, the technique is employed to anchor the stationary maxima or minima within the experimental design region of second-order regression models (Liyana-Pathirana and Shahidi, 2005). Factors were constrained to stationary points ranging from the centre point of the design matrix (i.e., $X_1 = X_2 = X_3 = X_4 = 0$) to the maximum perimeter of a sphere of radius 1.00, in accordance to the nature of the BBD matrix. Presented in **Table 3.8** is the printout of the ridge analysis of maximum response carried out using the RSREG procedure.

Table 3.8. An estimated ridge of maximum response for variable CBP using the RSREG procedure.

Coded Radius	Estimated Response *CBP*, mg g^{-1}	Standard Error	Uncoded (Natural) Factor Values			
			T, °C	*Time*, mins	*pH*	*LS*, ml g^{-1}
0.00	29.46	7.43	70	6.00	2.0	30.0
0.10	32.93	7.41	72	6.03	2.0	29.2
0.20	36.75	7.35	73	6.05	1.9	28.4
0.30	40.93	7.26	74	6.07	1.9	27.6
0.40	45.49	7.15	76	6.08	1.9	26.7
0.50	50.43	7.06	77	6.09	1.8	25.9
0.60	55.76	7.01	78	6.09	1.8	25.1
0.70	61.48	7.05	79	6.09	1.7	24.3
0.80	67.61	7.21	80	6.09	1.7	23.6
0.90	74.14	7.55	81	6.09	1.6	22.8
1.00	81.09	8.10	82	6.08	1.5	22.0

It is clear from the table, as it is from the adjoining response–radius and factor–radius plots (**Figs. 3.5a** and **b**), that the magnitude and propagation of CBP is congruent with the previous claim that the absolute maximum product obtainable from this process might not be within the experimental region. Hence the most reasonable candidates at the design radius of two units are: X_1 = 0.4002; X_2 = 0.0210; X_3 = -0.4526; and X_4 = -0.7966 in the design (coded) units, which translate to T = 82 °C, time = 6.08 min, pH = 1.5 and LS = 22.0 ml g^{-1} in their natural symbols and units. Interestingly, the ridge plot (**Fig. 3.5b**) reaffirmed that, from the centre points, while temperature was positively related to the response, both solvent properties (pH and LS) were negatively related. Extended MW irradiation beyond 6 min, however, bestowed no significant effect on the CBP obtained.

3.3.5 Regression model validation

Verification experiments performed at the predicted conditions derived from ridge analysis of RSM showed that experimental values were reasonably close to the predicted values validating the overall adequacy of the predicted models. Moreover, the verification experiments also proved that the predicted values for the model with cranberry pomace could be satisfactorily achieved within 95% confidence interval of experimental values (**Table 3.9**). For the purpose of technical feasibility, system and solvent conditions were slightly amended as noted in the table. **Table 3.9** also indicates that the CBP recovered, 83.3±1.6 mg g^{-1} (about 8.33 **wt**% db) at these conditions accorded within the experimental error given.

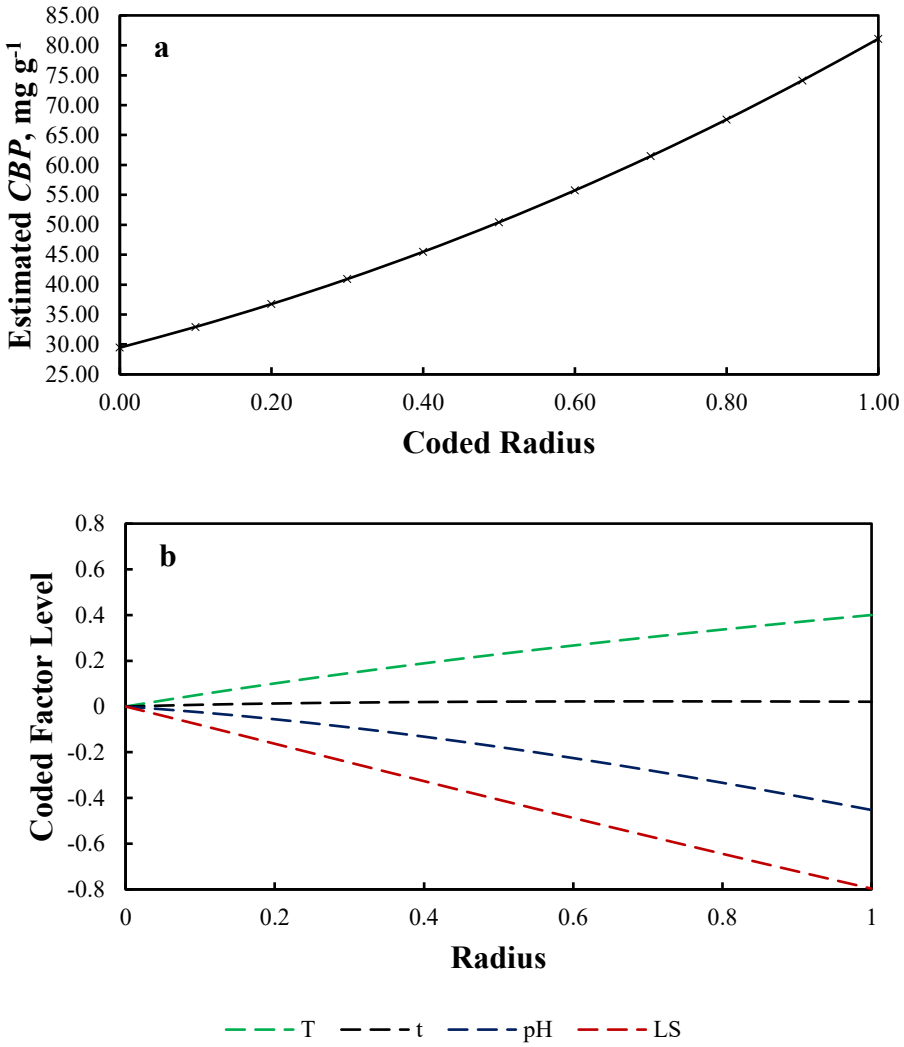


Figure 3.5. Ridge plots of the predicted response surface.

Table 3.9. Validation of optimality at predicted process conditions.

Process	Optimum condition				Yield, mg g^{-1}
	T, °C	*Time*, min	*pH*	*LS*, ml g^{-1}	
Predicted	82	6.0838	1.5473	22.0343	81.09
Experimental	82	6.08	1.54	22.03	83.3±1.6 [a]

[a] values are standard deviations of triplicate determinations.

3.3.6 Comparison with conventional extraction method

At conditions close to those obtainable in the industry, yield of pectin from cranberry pomace is reasonably high (**Fig. 3.6**). Importantly, the only adjusted factor, solvent to biomass ratio (*LS*), had an effect on the output of pectin in the traditional extraction process. Unlike in MAE, there was a dichotomy in the effect that *LS* has on the pectin output: an initial 62% improvement in the yield from *LS* 20:1 to 30:1 is followed by ca. 20% decline in the response. One presumable explanation for this phenomenon is that the hydrolysis events that typically

characterize pectin extraction are stochastic. By implication, there is a high level of polydispersity in the molecular sizes; in environments where the chains are allowed to spread out (as in high solvent ratio), chances of the smaller molecular sized polymers being separated from those with higher degree of polymerization. **Figure 3.6** also shows that yield of pectin at optimum MAE operation was more than 17% better than the best yield from conventional heat extraction. A yield of 8.33% for MAE, as against 7.08% from CHE, was a product not only of a shorter process time (~6 vs 60 min), but also of reduced solvent (22 vs 30 vol/wt) and temperature/energy (82 vs 90 °C) requirements. However, it should be said that the superior acidity of the solvent used in MAE relative to the CHE (*pH* 1.54 vs 2.00) connotes a marginal cost increase to the former process in the form of the amount of acid required to achieve *pH* of that magnitude.

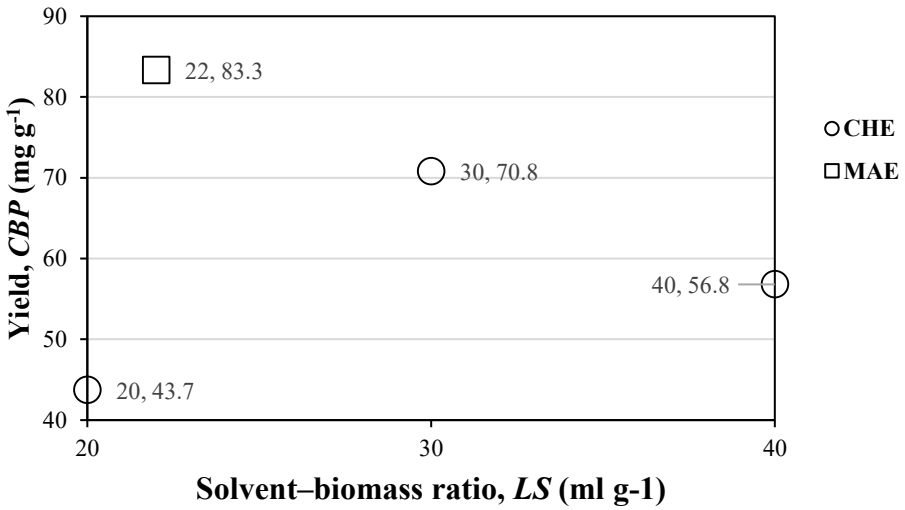


Figure 3.6. Comparison of cranberry pectin yields obtained under three CHE conditions with the optimized MAE process.

The lower operating temperature required by the MAE process can be expressed in estimated energy savings terms using the heat energy equation (**Eq. 3.5**).

$$Q = mc\Delta T \qquad \text{Eq. 3.5}$$

where: m is the total mass of dispersion of solvent and biomass (in g); c, the specific heat capacity of the solvent–CB pomace dispersion (in J kg^{-1} K^{-1}); and ΔT, the change in temperature (in K). For a 1 g biomass sample, CHE requires 30 ml of solvent (or ~30 g), while MAE requires 22 ml (or ~22 g), resulting in 31 and 23 g of heated matter, respectively. Also, from

an initial temperature of 24 °C, respective ΔT's are 66 and 58 K. Computations using Equation 3.5 show that CHE requires 48.4% more energy relative to MAE, under the assumption of identical sample specific heat capacity, to raise the temperature of the sample to that required for the entire duration. This is excluding the energy consumption while maintaining the system during the entire process duration, which again is expected to be higher for CHE than MAE.

3.4 Concluding remarks

Cranberry pomace was explored as a potential biomass for physicochemical transformation. Utilizing it for pectin production seems attractive, considering the sizeable amount of yield obtained. Herein, not only was the quantitative and temporal mass transfer of the compound examined, the influence of microwave energy on the output was assessed, with two system-related (namely, temperature and hydrolysis time) and two solvent-related (namely, solvent–biomass ratio and pH) properties examined. Their interactions among these parameters were estimated, particularly as they impact upon the dry mass of extracted pectin; this was effectively performed by response surface methodology. Temperature and solvent–biomass ratio immediately proved significant in defining the yield of pectin, with their increasing magnitudes generally playing positive and negative roles on the yield, respectively. Increasing irradiation time did not seem an important factor. Following ridge analysis, an optimum yield of 8.33 wt% was obtained under conditions: temperature, 82 °C; irradiation time, 6.08 min; solvent-biomass ratio, 22.03 ml g^{-1}; and solvent pH, 1.54. Finally upon comparison, extraction performed using microwave heating requires less energy and resulted in up to 17% better pectin yield than that by conventional heating.

Connecting text

In the preceding chapter, microwave-assisted extraction of pectin from cranberry pomace was investigated so as to understand the effect of varied temperatures (40–100 °C), times (2–10 min), and solvent properties (pH 1–3; and solvent–biomass ratio, 20–40 ml g^{-1}) on the yield obtained. The extraction process was optimized using the response surface methodology, and a quadratic model was developed. Comparisons of the effects of MAE and conventional heating extraction on pectin yields and other parameters were also drawn. In the next part, chemical, macromolecular, and rheological assessments of cranberry pectin properties are undertaken, which will in essence help to ascribe potential functionality to the biopolymer, in a number of in-solution food and non-food applications.

4. CHEMICAL, MACROMOLECULAR, AND RHEOLOGICAL PROPERTIES OF PECTIN FROM CRANBERRY POMACE

Abstract

Chemical and structural properties like galacturonic acid, degree of methyl esterification and antioxidant activity, as well as colour of pectin extracted from cranberry pomace have been assessed. Macromolecular properties including intrinsic viscosity ([η]) and viscosity-average molecular weight (M_v), as well as the flow and viscoelastic behaviour of aqueous pectin solutions were also studied. The results indicated that the antioxidant cranberry polysaccharide obtained had a medium uronic acid content (43–52%) which depended on extraction method used. Furthermore, the pectins obtained by either method were low methoxyl pectins. The magnitudes of [η] and M_v were determined as 2.15 dl g^{-1} and 39 kDa, respectively. Dispersions of the polysaccharide within the concentration range of 1.0–4.0 wt% did exhibit pseudoplastic flow behaviours. However, steady shear viscosity only coincided with complex viscoelastic parameters at values of concentration $c \leq 2.0$ wt%. Findings from this study leads to potential uses of this novel polysaccharide as an antioxidant dietary fibre, a food thickener, and a stabilizing agent. It was therefore concluded that not only is microwave-assisted extraction of cranberry pectin an efficient technique, but pectin recovered in such a way presents rheological and solution behaviour that are attractive for a number of food applications, albeit at high solid concentrations.

Keywords: polysaccharide characterization; FT-IR; steady shear flow behaviour; viscoelasticity; pectin; cranberry

4.1 Introduction

Hydrocolloids, which include most polysaccharides and certain proteins, have far-reaching applications within the food industry and elsewhere. With applications ranging from viscosity modification, to gel initiation, texturizing, binding and sugar/ice crystal growth inhibition, it is clear that these macromolecules are frequently called upon. This is evidenced in their regular appearance on the ingredient lists of many food and related products. It has, however, been established that much (if not all) of these functionalities are directly linked to the structure and certain intrinsic properties of these polysaccharides.

Pectin is one of the most renowned hydrocolloids and is naturally found in the cell wall/middle lamella of most terrestrial flora as a hetero-polysaccharide, chiefly containing α-(1→4)-linked galacturonic acid (GalA) as its main monosaccharide. *In situ*, pectin serves as a support to the cell wall of plants and is found to be chemically linked to cellulose, hemicellulose, and even protein moieties (Ridley *et al.*, 2001). For commercial purposes, it is harnessed (mostly from citrus and apple processing by-products) as a natural renewable polymer for the purpose of not only meeting some of the functional uses mentioned above, but likewise, increasingly, for its nutritional contribution as a dietary fibre. Novel sources of pectin are being explored, including waste streams obtained from processing olive (Rubio-Senent *et al.*, 2015), gold kiwifruit (Yuliarti *et al.*, 2015), mango (Berardini *et al.*, 2005), tomato (Grassino *et al.*, 2016), and *Aloe vera* (Gentilini *et al.*, 2014); pectins from some of these unconventional sources have presented appealing physicochemical properties which could have positive implications on their functionalities.

Physicochemical characterization of polysaccharides is an important first step towards a number of downstream scientific and industrial functions; moreover, successful process optimization and development currently require both a knowledge and adequate control of molecular details (Rußler *et al.*, 2012). Numerous studies on the structure of pectins (**Fig. 4.1**) exist. It is known that they are acidic, and in addition to the preponderance of GalA, varying degrees of neutral sugars (xylose, glucose, arabinose, etc.) and even ferulic acid have been confirmed along their chains, which stem from the rhamnose interruptions along the main backbone. Extraction techniques and conditions, plant species, and quantification technique all influence the amount of these monosaccharide groups in pectin (Yuliarti, 2011). Two classes of pectin on the bases of the degree of methyl esterification (DE) of its galacturonic acid units, namely low methoxyl (LM) and high methoxyl (HM) with DE less than and greater than 50%, respectively. Additionally, pectin differ in their content of the GalA based on their source, thus

prompting regulations regarding acceptable GalA: 65% for food uses and 74% for pharmaceuticals (EC, 1996; FAO/WHO, 2009; FCC, 2012). Pectin is a highly-branched polymer. This translates to the relatively high molecular weight of pectin often reported (M_w 20–200 kDa) which is comparable to other polyelectrolyte hydrocolloids, carrageenan (300–500 kDa), and alginate (32–400 kDa) but much lower than xanthan gum (2 000–20 000 kDa) (García-Ochoa *et al.*, 2000; Harding *et al.*, 1991; Lecacheux *et al.*, 1985; Rinaudo, 2008). It follows that vagaries of species and recovery techniques translates to the polydispersity that is associated with the molecular size distribution of pectins.

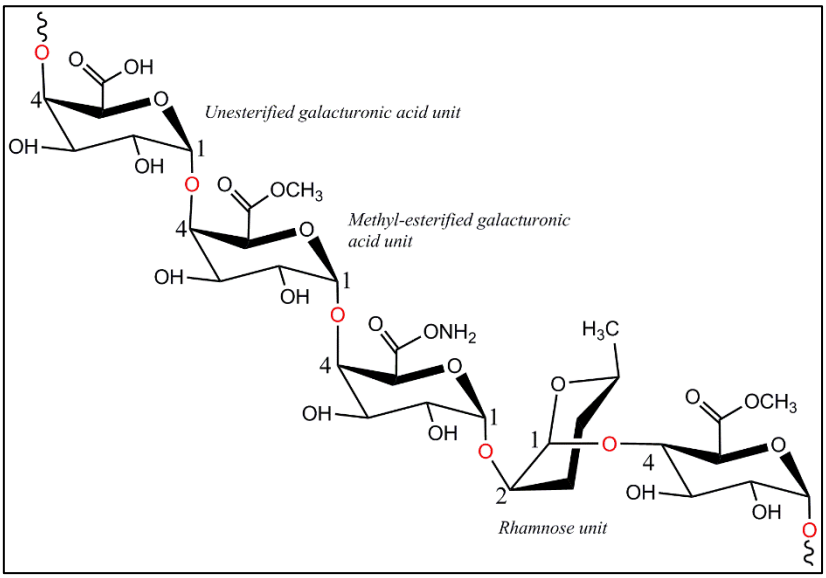


Figure 4.1. Pectin structure showing methyl esterified and non-methyl-esterified galacturonic acid units.

Rheology of different classes of pectins has been studied both in solutions including dilute solutions and gels. Being polyelectrolytes, ionic aqueous salt solutions are considered theta (θ) solvents used in the study of the conformational behaviour of pectin at low concentrations (Rao, 2014a). Gelation of pectin, like in most polysaccharides, has been well established as involving the formation of stable intermolecular 'junction zones' between structurally and conformationally regular chain sequences, but with some differing nuances in the mechanism of junction zone-formation for LM and HM classes of pectin. HM pectins gel in a condition of low water activity (pH 3. And co-solute such as sucrose), while for LM variants, a complexation involving divalent cations from some salts (commonly Ca^{2+}) creates the much needed junction zones.

Earlier, cranberry bagasse was explored for its pectin using microwave (MW) energy, and it is

therefore the aim of this study to unravel some of the chemical, macromolecular, and rheological properties of MW- and conventionally-extracted cranberry pectin (CBP), with a view of identifying potential niche applications in food and other aqueous systems. Specifically, the monosaccharide (GalA) content, antioxidant activity, intrinsic viscosity, molecular weight, and flow and viscoelastic behaviours are to be assessed using appropriate techniques. For the viscoelastic behaviour, the focus was largely on the effect of polymer concentration leading to a better understanding of the polymer's chain dynamics in the semi-dilute regime.

4.2 Materials and Methods

4.2.1 Materials

Pectin (CBP) was recovered from cranberry pomace using both the conventional heating extraction (CHE) method and an optimized microwave-assisted extraction (MAE) process, as described in **Chapter 3**; obtaining CBP involved a pre-treatment stage, an extraction step and a purification routine. Briefly, cranberry (*Vaccinium macrocarpon*) fruits were washed, juiced and the pomace (containing seed, pulp, stem and skins) was converted into dry powder after going through some pre-treatment stages, including washing, oven-drying, milling, sieving and packing in Ziploc bags. For MAE optimum conditions were: temperature, 82 °C; time, 6.08 min; pH, 1.54; and solvent to biomass ratio, 22.03 ml g^{-1}. CHE extraction proceeded at 90 °C for 1 h, using solvent conditions: pH 2 and solvent to biomass ratio (30.00 ml g^{-1}).

Commercial citrus pectin and poly galacturonic acid were obtained from Sigma Aldrich (Ontario, Canada), having characteristics as shown in **Table 4.1**. All reagents used for the chemical and other characterization, including sulfamic acid, potassium hydroxide, sulphuric acid, sodium tetraborate, *m*-hydroxyldiphenyl, sodium hydroxide, sodium chloride, DPPH*, ethanol, and ascorbic acid, were of analytical grade.

Table 4.1. Chemical information of pectins used in this study.

Sample	GalA (%)	DE (%)
LM pectin [a]	79	8
HM pectin	–	≥ 85
Poly galacturonic acid (PGA)	~90	~0

[a] subsequently referred to as commercial citrus pectin (CCP).

4.2.2 Chemical and appearance analyses

4.2.2.1 Sugar quantification by UV–Vis Spectroscopy

The galacturonic acid (GalA) content of pectin was estimated by a colorimetric technique

known as sulfamate/*meta*-hydroxyldiphenyl method (Filisetti-Cozzi and Carpita, 1991; Melton and Smith, 2001). Concisely, sulphuric acid hydrolysis of pectic polysaccharides is followed by a colorimetric assay involving spectrophotometric absorbance measurement at a wavelength of 525 nm, using an UltroSpec 2100 pro (Biochrom Ltd., Cambridge, England). GalA contents were then estimated by reading off monosaccharide concentration on simultaneously produced calibration curves. The calibration curves were produced from D-(+)-galacturonic acids standard solutions at concentration 100–1500 µM. **Appendix B** provides more information on the the GalA content determination protocol as well as the standard curve.

4.2.2.2 Degree of methyl esterification by infrared spectroscopy

The degree of methoxylation (DE) of cranberry pectin were determined by IR spectroscopy, following the procedure by Kyomugasho *et al.* (2015). FT-IR-ATR analysis of pectin standards (whose DE values, in the range 0 to 85%, were theoretically determined by blending PGA and HMP gravimetrically) was performed using Nicolet iS5 ATR–FT-IR spectrophotometer operating in attenuated total reflectance (ATR) mode with 4 cm^{-1} resolution, and these were used in preparing the calibration curve. The analysis involved collecting 32 co-added scans of absorbance spectra across the 4000-400 cm^{-1} wavenumber range. Post-data collection analyses include smoothening, baseline correction, peak resolve (i.e. deconvolution), and peak area determination, which were all performed within the OMNIC® software. The Peak Resolve functionality in the software was necessary as the two peaks between 1800 and 1400 cm^{-1} overlapped and essentially involved using the convergence routine (specifically the Fletcher-Powell-McCormick algorithm) to plot two Gaussian profiles having initial peak centre values set at 1735 and 1650 cm^{-1}, respectively. The areas of both peaks were then determined as A_{1735} and A_{1650}, indicating the number of esterified and free carboxylic groups on the pectin chains, respectively. The ratio R_{DE} between the esterified area and the sum of esterified and free areas, i.e. $R_{DE} = \frac{(A_{1735})}{(A_{1735}+A_{1650})}$, is correlated with the percentage degree of methyl esterification $\%DE$ of the pectin, and the regression equation so obtained was:

$$\%\text{DE} = 255.84R_{DE} - 116.92,\ R^2 = 0.976 \qquad \text{Eq. 4.1}$$

4.2.2.3 Antioxidant activity

The antioxidant activity of extracted pectin was imperative for two reasons. First, being sourced from a polyphenol-rich raw material—cranberry, anthocyanin pigmentation persisted on the lyophilized cranberry pectin extract, even after rigorous pre-treatment and purification of the pomace and pectin, respectively (see **Section 4.2.2.4**) (Padmanabhan *et al*., 2016). Secondly

and more importantly, the multiplicity of hydroxyl groups found on pectin chain has seen the compound touted as possessing some antioxidant properties (Wikiera *et al.*, 2015). For this purpose, the DPPH• (1,1-diphenyl-2-picrylhydrazyl) inhibition assay, according to the accepted method (Molyneux, 2004; Wikiera *et al.*, 2015), was used with a little modification. In a nutshell, to 1 ml of freshly-prepared 0.2 mM DPPH• in ethanol solution was added 1 ml of aqueous pectin solution (0.4 % (w/v)). Absorbance of the resulting solution and blank (DPPH) solution were then collected at 516 nm against a blank background, after a 30 min incubation at room temperature in darkness. Ascorbic acid (Vitamin C) was used as positive control, and the capability of pectin to scavenge DPPH• radical was computed using the relation:

$$Scavenging\ activity\ (\%) = \left[\frac{A_0 - A}{A_0}\right] \cdot 100 \qquad \text{Eq. 4.2}$$

where: A_0 is the absorbance of DPPH• solution; and A, the absorbance of resulting solution (i.e., pectin (or control) plus DPPH• solution). Measurements were performed using UltroSpec 2100 pro UV–Vis spectrophotometer (Biochrom Ltd., Cambridge, England).

4.2.2.4 Colour measurement

The effects of the pre-treatment procedure (i.e., with or without washing step), as well as extraction technique (microwave heating or conductive–convective heating), on the physical appearance of extracted pectin was assessed using a pre-calibrated colorimeter, CR-300 Chroma-Meter (Konica Minolta, Japan). Three grabs were taken by the instrument's light projection tubes and averages were displayed for each of the colour parameters of the CIELAB colour space: L^* (lightness), a^* (redness and greenness), and b^* (yellowness and blueness); furthermore, colour values were presented as mean ± standard deviation of their equivalents from duplicate samples. Calibration were against a standard white tile ($L^* = 97.36$, $a^* = 0.09$, $b^* = 2.81$). Total colour difference (ΔE) was calculated as:

$$\Delta E = \sqrt{(\Delta L*)^2 + (\Delta a*)^2 + (\Delta b^*)^2} \qquad \text{Eq. 4.3}$$

where Δ denotes the difference between standard (white tile) and sample readings.

4.2.3 Macromolecular and hydrodynamic analyses

4.2.3.1 Capillary viscometry

The dilute solution behaviour of polymers give information about their conformation and sizes (i.e., hydrodynamic volume); this is because at such low concentrations, the polymer chains

are apart. For illustration, the intrinsic viscosity [η] depends on the dimension of the chain. [η] of a macromolecule supplies one of the most reflective information about its contribution to the viscosity of aqueous systems, and here, it was determined in line with standard practice for polymers (Rao, 2014a) as described below.

Being a polyelectrolyte, dilute dispersions of pectin were made in a high ionic strength solvent (i.e., aqueous solution of 0.1 M NaCl solution) (Lopes da Silva *et al.*, 1993). Stock solution of 0.5% (dry basis, w/w) was prepared by dispersing an appropriate amount of pectin powder in the solvent, and stirring for two hours (and/or until complete dissolution) at 40 °C. Dilution of stock was performed to obtain a concentrations range of 0.075–0.500% (dry basis, w/w). Zero-shear (capillary) viscosities, η and η_s for pure solvent (0.1 M NaCl solution) and for pectin samples solution (in the same solvent) were determined by capillary viscometry, using a calibrated CANNON–Fenske Routine Viscometer (size no. 150) maintained at 24 ± 0.1 °C. The relative viscosity, η_r, was computed as:

$$\eta_r = {}^{\eta}/_{\eta_s} = {}^{(t \times \rho)}\Big/_{(t_s \times \rho_s)} \qquad \text{Eq. 4.4}$$

where, t_s and t are the efflux time in seconds of solvent and solution between the two etched marks of the viscometers, respectively; and ρ_s and ρ, the densities of solvent and solution, respectively, which were assumed to be essentially equal giving the low concentrations used for the study. Huggins' and Kraemer's equations were plotted in order to extrapolate values of [η], using η_{sp}/c against c and $\ln(\eta_r)/c$ against c, respectively. This way, [η] represents the limiting value of either reduced viscosity (η_{red} or η_{sp}/c) or inherent viscosity ($\ln \eta_r)/c$ at infinite dilution of the polymer, as mathematically presented in **Equations 4.5 and 4.6**.

$$[\eta] = \lim_{c \to 0} \eta_{red} = \lim_{c \to 0} \frac{\eta_{sp}}{c} = \lim_{c \to 0} \frac{\eta_r - 1}{c} = \lim_{c \to 0} \frac{\eta - \eta_s}{\eta_s \cdot c} \qquad \text{Eq. 4.5}$$

$$[\eta] = \lim_{c \to 0} \eta_{inh} = \lim_{c \to 0} \frac{\ln \eta_r}{c} \qquad \text{Eq. 4.6}$$

An additional method of [η] determination, known as Fedors' equation (Fedors, 1979) (**Eq. 4.7**) was also employed to verify the combined Huggins' and Kraemer methods.

$$\frac{1}{2(\eta_r^{1/2} - 1)} = \frac{1}{[\eta]c} - \frac{1}{[\eta]c_m} \qquad \text{Eq. 4.7}$$

where c_m is a factor showing Fedors' concentration limit.

4.2.3.2 Viscosity-average molecular weight determination

Molecular weight of the pectin samples were estimated by viscometry, using constants K and α which are available in the literature for pectin macromolecules, as well as intrinsic viscosities determined as in **Section 4.2.3.1** above; the molecular weight values were obtained from the following empirical relation known as Mark–Houwink–Sakurada (MHS) equation:

$$[\eta] = KM_W^{\alpha} \qquad \text{Eq. 4.8}$$

where: $[\eta]$ is the intrinsic viscosity determined by capillary viscometry, dl g^{-1} (see **Section 4.2.3.1**); M_v, the viscosity-average molecular weight of the polymer, kDa; K and α are MHKS parameters taken respectively for pectin as 9.55×10^{-4} dl g^{-1} and 0.73 under studied polymer-solvent system (Anger and Berth, 1986). Efflux time used in the eventual intrinsic viscosity determination were based on triplicate measurements, so M_v are consequently a result of similar replications.

4.2.4 Rheometry

Rheological measurements were conducted using AR-2000 controlled-stress rheometer (TA Instruments). Cone-and-plate geometry of configurations, 40 mm diameter and angle = 2° was used to study polysaccharide dispersions. The plate gap was set to 1 mm. The geometry used had a solvent trap, and that, coupled with an insulating thermal cover, was used to minimize solvent loss through ambient evaporation by providing a vapour (saturated) barrier to seal the environment. Test samples were equilibrated for 2 min prior to each measurement. Meanwhile, thermal equilibrium (within ± 0.1 °C of set temperature values) of sample was ensured by a Peltier element equipped with the bottom plate of the unit, consisting of a submersible pump-powered water cooling system. All determinations were performed by imposing logarithmic torque ramp which helped decrease initial acceleration and the effect of instrument inertia.

4.2.4.1 Solution formulation

Monophasic solutions/dispersions of the different pectin (CBP and CCP) and wheat starch powders having mass concentrations, 1.0, 2.0 and 4.0 **wt%**, were prepared with 40 °C distilled water as solvent. Initially, dispersions were gently stirred (150 rpm) for 30 min at 40 °C using Incu-Shaker™ Mini shaking incubator (Benchmark Scientific, Inc., NJ, USA), followed by an overnight stirring at ambient temperature; this practice aided proper dissolution and hydration of the polysaccharides.

4.2.4.1 Steady-state flow measurements

The flow behaviours of semi-dilute/concentrated pectin dispersions were studied by

performing a steady-shear procedure within the shear rate range of 0.01–1 000 s^{-1}. Elucidation of their specific flow behaviour was assured by fitting flow parameters into the power-law model of Ostwald-de Waele, given as:

$$\tau = k\dot{\gamma}^n \quad \text{Eq. 4.9}$$

where τ is the shear stress (Pa); $\dot{\gamma}$, the shear rate (s^{-1}); k, the consistency index ($Pa \cdot s^n$), whose numerical value is related to concentration or liquid consistency; and n is the dimensionless flow behaviour index, whose value demonstrates the extent to which a liquid departs from a Newtonian behaviour.

4.2.4.2 Small-amplitude oscillatory shear (SAOS) test

Viscoelastic behaviours of the polysaccharide dispersions were assessed by performing a frequency sweep in the frequency range of 0.01–10 Hz (or angular frequency range of 0.06–62.83 rad s^{-1}) and within the linear viscoelastic region (LVR) of the test sample. LVR of the gum solutions was established by performing an amplitude (strain) sweep test Data were simultaneously transformed, using TA Rheology Advantage data analysis software (v 3.0.24, TA Instruments, Newcastle, UK), into frequency-dependent material metrics used to characterize the system viscoelastic properties, namely complex modulus G^*, its real and imaginary components, G' and G''—respectively referred to as storage modulus and loss modulus—and phase angle, $\delta = \tan(G''/G')$.

4.2.5 Statistical analysis

Except otherwise stated, data from all experiment were analysed with one- or multi-way analysis of variance (ANOVA) using JMP software (v 11.2.0, SAS Institute Inc.). A probability $p < 0.05$ was selected as a benchmark for determining significant results; differences among treatments were determined as using a Tukey test. Finally, data were reported as mean ± SD of duplicate or triplicate determinations.

4.3 Results and Discussions

4.3.1 Chemical properties

4.3.1.1 Galacturonic acid (GalA).

The GalA contents of extracted pectin differed significantly ($p < 0.05$) with the extraction method used as shown in **Table 4.2**. Pectins of cranberry pomace heated by microwave had a lower GalA units (43.4%) than those resulting from conventional heating (47.1%). It is expected that the extended extraction duration employed in CHE results in greater degradation

of neutral side chain, which results in a higher fraction of galacturonic acid sugar found on individual chains. As irradiation time is lower for MAE, the said neutral sugar degradation is reduced. Additionally, it was found that GalA content of cranberry pectin extracted by conventional heating (i.e., C-CBP) increased with increasing solvent to biomass ratio (from 45.5% for 20 ml g^{-1} to 51.4% for 40 ml g^{-1}).

Table 4.2. Effect of extraction method on galacturonic acid content and degree of methyl esterification of cranberry pectin.

Extraction method	GalA content	DE
CHE	47.1 ± 1.9	21.1 ± 5.0
MAE	43.4 ± 2.3	25.9 ± 4.6

4.3.1.2 Degree of methyl esterification (DE)

The Fourier Transform Infrared (FT-IR) spectrum of microwave extracted cranberry pectin (CBP) was examined in order to identify the functional groups present on the polysaccharide chains in contrast to low and high methoxyl citrus pectin samples (**Fig. 4.2**). It is noted that the spectra are very similar. The broad peaks from 4000 to 3000 cm^{-1} are due to O–H stretching, while the smaller peak which almost overlaps the first one (with highest point around 2900 cm^{-1}) are attributed to C–H stretching. Also evident are the presence of the carbonyl functional groups (C=O) on both methyl-esterified ($–COOCH_3$) and non-methyl-esterified (–COOH) carboxylic acid, which are observable around peaks 1735 and 1650 cm^{-1}, respectively (Kyomugasho *et al.*, 2015). The intensities of these peaks were higher for the citrus pectins, probably due to their superior galacturonic acid content (e.g. 79% in the LM citrus pectin vs ~50% in CBP). The regions from 1300 to 800 cm^{-1} are considered the fingerprint regions for the samples, within which the peak in the region 1300 to 1000 cm^{-1} is attributed to C=O stretching, and observable differences are difficult to decipher (Nep *et al.*, 2016).

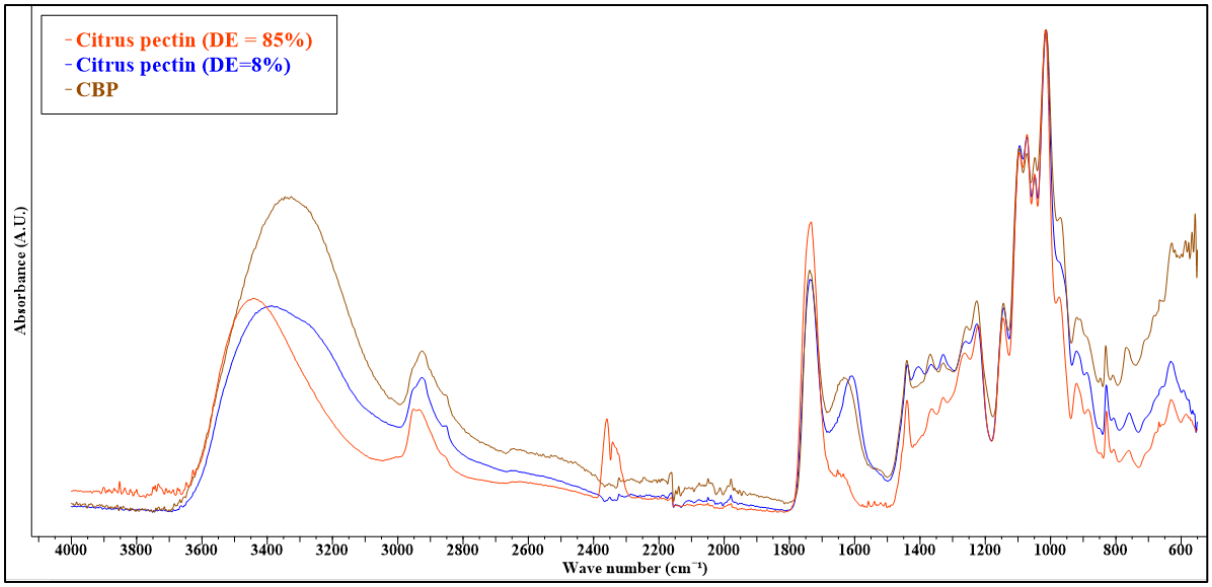


Figure 4.2. Infrared spectra of citrus and cranberry pectins.

Table 4.2 also presents the DE of pectin from cranberry, extracted using conventional heating and microwave energy. DE of pectin obtained by both methods were not significantly different ($p > 0.05$). Whereas Yuliarti (2011) used capillary electrophoresis to assess the DE of pectin from gold kiwifruit, reporting values as high as 90% (i.e. high methoxyl pectin), pectin obtained from cranberry were of the low methoxyl (LM) variant. It has previously been reported that certain fruit and vegetable by-products are natural sources of LM pectin, including those from: passion fruit, *Passiflora edulis* f. flavicarpa Degener (Yapo and Koffi, 2006); peach (Pagán *et al.*, 2001); sunflower head (Hua *et al.*, 2015); and cashew, *Anacardium occidentale* L. (Yapo and Koffi, 2013). The obtained LM pectins are understood to result from degradation of nascent HM pectin, following the activity of the enzyme pectin methylesterase (PME) during storage (Pagán *et al.*, 2001; Yapo and Koffi, 2013). Prolonged heating exposure is also known to cause de-esterification.

4.3.1.3 Antioxidant activity.

DPPH$^{\bullet}$ scavenging assay is a choice procedure for determining the *in vitro* antioxidant activity of compounds, including food systems, due to its simplicity and minimal solution preparation time. In this assay, donation of H^{+} to a DPPH• radical by a typical antioxidant results in an observable colour change from purple/red to yellow which is amenable to quantification by absorbance measurement at wavelengths around 516 nm (Brand-Williams *et al.*, 1995; Rha *et al.*, 2011). **Figure 4.3** presents the antiradical capacity of cranberry pectins extracted by conventional heating (C-CBP) and microwave heating (M-CBP) relative to a commercial citrus

pectin (CCP). Cranberry pectin resulting from the optimized microwave-assisted extraction (described earlier) demonstrated significantly higher ($p < 0.05$) inhibitory activity towards DPPH• free radical formation than both CCP and C-CBP. Further, of the three tested pectins, only M-CBP had half-maximal (50%) inhibitory concentration (IC_{50}) that was evidently below the 4 mg ml^{-1} used in this study (mean values); this implies that greater amount of CCP and C-CBP are required to effect the same scavenging activity as M-CBP. The shorter process time and marginally lower temperature of the MAE process could have minimized the degradation of the persistent anthocyanins (and other polyphenols) attached to its polysaccharides. Although not known with certainty, comparable conditions to those used for C-CBP recovery were conceivably employed in the production of CCP; at such temperatures chemical instabilities resulting in the form of degradation of heat-labile phenolic compounds are not uncommon. Chemical properties (GalA, DE, neutral sugars) and molecular weight of pectin have also been identified as possibly having effects on the antioxidant activity of pectin. The antioxidant activity of CBP thus increases its importance as a probable novel source of natural additive, particularly in relation to the demonstrated correlation between ingestion of antioxidant-rich foods and the suppression of human diseases (Lu and Yeap Foo, 2000; Sellimi *et al.*, 2015). Similar antioxidant dietary fibre has been recovered from Alperujo, the semi-solid by-product of the olive oil industry (Rubio-Senent *et al.*, 2015).

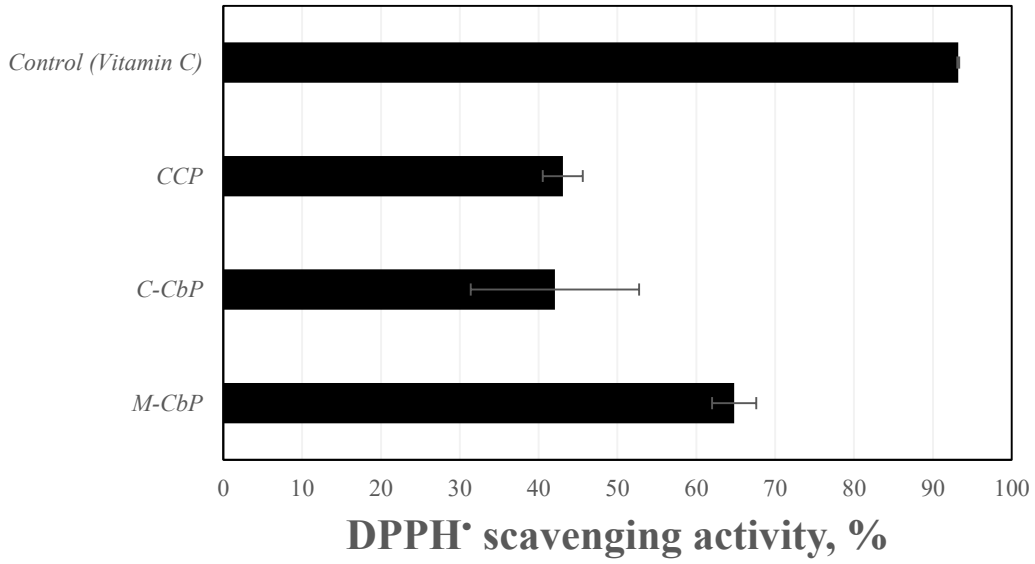


Figure 4.3. The *in-vitro* antioxidant activity of pectin extract relative to reference compound. [CCP, C-CBP, and M-CBP are commercial citrus pectin, CHE-extracted pectin, and MAE-extracted pectin, respectively, error bars represent standard deviations, and all samples are at 4 mg ml^{-1} concentration].

4.3.1.4 Colour of extracts.

The colour of food and allied products, including ingredients, is an extremely sensitive quality of the product, as it is one of the prime observation of customer appeal, and its state informs appeal or otherwise. Pectin is often added to food systems in conditions where its "colour-enhancing properties" are unrequired. Colour measurement data for dried extracts from two differently pretreated biomasses (washed and unwashed) are presented in **Table 4.3**. The effect of extraction method—conventional heating (CHE) or microwave heating (MAE)—is also captured in the table, in comparison with a commercial citrus pectin (CCP). Pectins recovered by CHE (C-CBP1 through C-CBP3) tended to exhibit increasing lightness (L^*) and decreasing ΔE with increasing solvent–biomass ratio (20 to 40 ml g^{-1}, respectively). This is expected, as more solvent volume implies more extract volume, which in turn means more alcohol use and availability (during precipitation and washing of extract) for essentially the same content of anthocyanin pigment. The observed decrease in redness (positive a^* values) of extract corroborates this assertion.

Table 4.3. Colour values ($L^*a^*b^*$)[a] of pectin resulting from different extraction routes.

Sample ID	L^*	a^*	b^*	ΔE[b]
Conventional heating				
C-CBP1	66.72 ± 1.07[c]	+23.77 ± 2.37	+13.74 ± 1.39	40.25 ± 2.59
C-CBP2	66.95 ± 0.57	+22.80 ± 1.03	+12.13 ± 0.34	39.09 ± 0.24
C-CBP3	74.25 ± 6.36	+20.87 ± 4.21	+9.98 ± 3.05	31.93 ± 8.00
C-CBP(Unwashed)[d]	43.03 ± 2.07	+40.10 ± 2.52	+21.70 ± 2.96	44.78 ± 1.45
Microwave heating				
M-CBP	58.29 ± 0.41	+21.05 ± 3.44	+8.60 ± 1.57	28.27 ± 1.13
Commercial				
CCP	82.36 ± 0.51	+2.59 ± 0.19	+26.63 ± 1.26	70.11 ± 3.33

[a] L=100 (white) to 0 (black); a = + (red) vs - (green); b = + (yellow) vs - (blue)
[b] Total colour difference
[c] Mean ± standard deviation
[d] obtained by CHE (under conditions: *T*, 80 °C; *Time*, 1.5 h; *pH*, 2; *LS*, 30 ml g^{-1}) from unwashed dry pomace.

Pectin from MAE was significantly darker ($p < 0.05$) than those from all CHE processes with similar biomass pretreatment techniques. Its L^* value (58.29) was closer to that of C-CBP (Unwashed) (43.03) than were C-CBP1 through C-CBP3 (66.72-74.25). This, however, highlights that the washing pretreatment step could have a positive sensorial effect for food applications of CBP, as was noted for pectin from tomato waste (Grassino *et al.*, 2016). Unwashed biomass yielded pectin with generally higher red and yellow coloration, as evidenced by its higher a^* and b^* values, compared to those of washed samples; indeed, the

tested sample was representative of the entire lot irrespective of the CHE process conditions (data not shown).

4.3.2 Intrinsic viscosity and viscosity-average molecular weight

4.3.2.1 Intrinsic viscosity ([η]).

Determination of intrinsic viscosity was conducted using isoionic dilutions of polysaccharide solution in 0.1 M NaCl, with concentration range 0.075–0.5 **wt%** and relative viscosity range of $1.1 < \eta_r < 2.8$, spanning the range suggested by Rao (2014a)—i.e., 1.2–2.0. As was observed in a number of polysaccharides, such as *Adansonia digitate* leaves polysaccharide (Nwokocha and Williams, 2016a), extrapolated intercepts of the combined Huggins' and Kraemer's plots do not always meet at $c = 0$. This was the case for CBP, and a common way around this is to report the average value of their y-intercepts as [η]. Such combined plot for CBP is presented in **Figure 4.4a**, and the two regression equations depict an intrinsic viscosity of 2.15 ± 0.05 dl g^{-1}. This value agrees well with the 2.12 dl g^{-1} obtained as the reciprocal of slope of the Fedors' plot (i.e., slope =1/[η]) in **Figure 4.4b**. Similar agreement existed for the low methoxyl pectin used in this study ([η] = 1.06 ± 0.03 and 1.06 dl g^{-1}, respectively). Correlations between [η] obtained by both the Huggins', Kramer's and Fedors' plots have previously been reported for a number dispersions containing modified and unmodified polysaccharides (Nwokocha and Williams, 2016a, 2016b; Rotureau, Dellacherie, and Durand, 2006). The parameter c_m in Fedors' equation is considered as the limiting concentration of polymer in solution and is deduced from the intercept of the regression plot (intercept = $1/c_m[\eta]$); for CBP, this value was 1.75 g dl^{-1}.

In addition, it has been reported that the Huggins' (K_H) and Kraemer's (K_K) constants are indicative of the polymer–polymer and polymer–solvent interaction within the system; indeed, values of K_H ranging from 0.3 to 1.0 represent conditions in good to theta (θ) solvents, respectively (Rao, 2014a). K_H for CBP dispersion was calculated from the slope of the Huggins' plot (slope = $K_H[\eta]^2$) as 0.68; the value of K_K was similarly deduced as 0.04 from the slope (slope = $-K_K[\eta]^2$ of the Kraemer's plot. Moreover, the theoretical relationship between these two constants ($K_H = K_K + 0.5$) was rather $K_H = K_K + 0.64$ in this case. Intrinsic viscosity values obtained for CBP is below the range (2.69–4.27 dl g^{-1}) reported for number of commercial low methoxyl and amidated low methoxyl pectins. Macromolecular properties of polysaccharide from *A. digitata* L., an African plant whose leaves are used as soup thickener and vegetable in West Africa, was examined under similar solvent conditions described here; values of intrinsic viscosity obtained was 3.27 dl g^{-1} (Nwokocha and Williams, 2016a), which

is again higher than that of CBP. The authors reported the presence of dissociated carboxylate groups and linked this to a possible presence of uronic acid as typical of anionic polysaccharides.

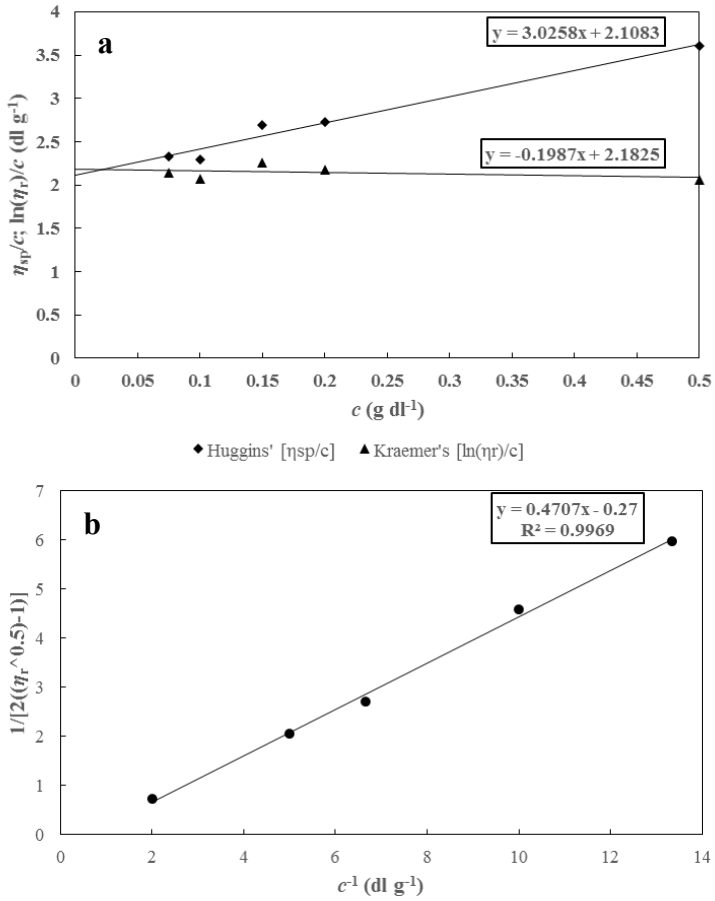


Figure 4.4. The intrinsic viscosity of cranberry (*V. macrocarpon*) pectin dispersion, with 0.1 M NaCl solution as solvent, obtained by: (a) Combined Huggins' and Kraemer extrapolations; and (b) Fedors equation method.

4.3.2.2 Viscosity average molecular weight (M_v).

Estimation of an index of molecular weight was based on the Mark–Houwink (MH) equation, using MH parameters previously reported by for pectin dispersions under similar conditions to those used in the present study (*solvent*: aqueous solution of 0.09 M NaCl; *temperature*: 25 °C). For CBP, M_v was 39.12 kDa, while CCP was found to be 14.85 kDa.

4.3.3 Rheological behaviours

4.3.3.1 Flow properties—effect of concentration

The viscous properties of aqueous solutions of CBP at concentrations ranging from 1.0 to 4.0

wt% were studied by performing steady-shear flow test over shear rate range 0.01 to 1000 s^{-1}, after which rheological data were fitted to a linearized power-law model:

$$\log \tau = \log k + n \log \dot{\gamma} \qquad \text{Eq. 4.10}$$

with parameters as described in **Equation 4.8**. Flow curves describe the relationship between shear stress (usually the ordinate, τ) and shear rate (usually the abscissa, $\dot{\gamma}$) responses of fluid systems. When the relationship is linear, the test fluid is said to be a Newtonian fluid, whereby 'apparent' viscosity remains the same over the range of shear rate. However, many fluid foods, including dispersion systems involving food hydrocolloids, exhibit non-linear log τ–log $\dot{\gamma}$ relationships (non-Newtonian fluids) (Ma *et al.*, 2014). Under such circumstances, defining viscosity requires a statement not only about the temperature, but also about the test shear rate. Moreover, these non-Newtonian fluids could be dependent or independent of time. Many polysaccharide dispersions have been shown to specifically exhibit time-independent flow behaviours that are shear-thinning (i.e., pseudoplastic) (Rao, 2014b). As noted earlier, the index n is used to classify fluid types, such that: for $n = 1$, the test fluid is Newtonian; for $n < 1$, fluid is pseudoplastic; and for $n > 1$, fluid is shear thickening (or dilatant).

Flow curves of aqueous CBP solutions at three concentrations ranging from 1.0 to 4.0 **wt%** are shown in **Figure 4.5**a with log τ vs log $\dot{\gamma}$ plotted in logarithmic scale and covering five decades of shear rates. Owing to increased intermolecular interactions between adjoining CBP molecules, one observes an increase in shear stress with increasing CBP concentration. The model parameters obtained from fitting the model into experimental data are summarized in **Table 4.4**. In the table, identical parameters for HM pectin solutions as well as wheat starch (WS) dispersions, at similar concentrations, were also presented for comparison. As **Table 4.4** suggests, all CBP solutions exhibited pseudoplastic liquid behaviour with flow behaviour index n less than unity, whose shear stress increases with polymer concentration. A general increase in pseudoplasticity with concentration, heralded by a decline in the magnitude of n, is exhibited by the solutions. In addition, consistency index k, increased with concentration of CBP, which is an expected observation as this quantity is linked to the polymer concentration/viscosity (Marcotte, Taherian Hoshahili, and Ramaswamy, 2001). It is believed that the disruption of CBP chain entanglement as solution is sheared is the main contributor to the decreased apparent viscosity as shear rate increased (Ma *et al.*, 2014). Indeed, solutions of pectin from pomelo (*Citrus maxima*) (0.4–2.0% (w/v)) also displayed shear-thinning behaviours, which was ascribed to the shear-induced conformation rearrangement that the pectin chains experience

during measurement (Methacanon, Krongsin, and Gamonpilas, 2014).

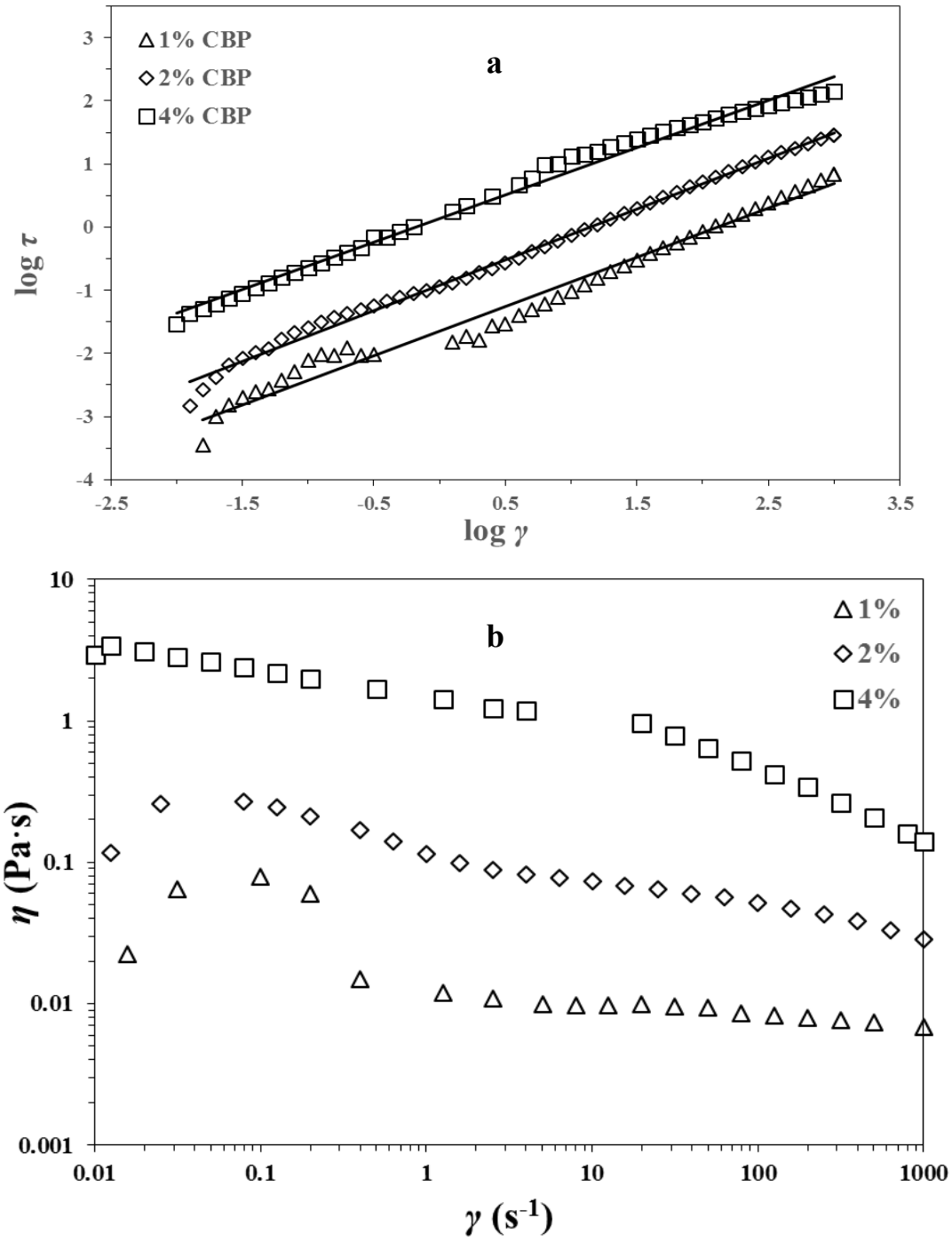


Figure 4.5. (a) Steady-shear flow behaviour curves of CBP solutions ranging from 1.0 to 4.0 **wt**% at 24 °C. Solid lines represent linear fit based on the power-law model of Ostwald-de Waele. (b) Viscosity as a function of shear rate for the same CBP solution concentration range at 24 °C.

This apparent viscosity–shear rate (η–$\dot{\gamma}$) relationship for CBP solution was studied at the same concentration range (1.0 to 4.0 $\mathbf{wt}\%$) at 24 °C. Although shear-thinning is the ideal behaviour of pseudoplastic fluids such as polysaccharide solution, yet as **Figure 4.5b** suggests, CBP solutions at all studied concentrations exhibited an initial shear-thickening behaviour, wherein the apparent viscosity increased with increasing shear rate, followed at some unique/critical shear rate ($\dot{\gamma}_c$) by momentary plateau region (i.e. the Newtonian plateau region). Equally noteworthy from the figure is the fact that this unique shear rate shifts to a lower magnitude with increasing CBP concentration. These Newtonian plateau can be characterized by an almost constant viscosity of great practical relevance, known as zero shear rate viscosity (ZSV), which, for example, dictates the ability of a polymer to act as a suspending agent. Similar observations were made by Ma *et al.* (2014) in sodium alginate solutions at the concentration range 1.0 to 3.0% (w/v). The authors reported an initial shear-thickening and later shear-thinning behaviour intermediated by a brief Newtonian plateau, which commenced and ended at a 'first' ($\dot{\gamma}_{c1}$) and 'second' ($\dot{\gamma}_{c2}$) critical shear rates. Carboxyl methyl cellulose (another food hydrocolloid) solutions have also been reported as having these three distinctive solution behaviour. This shear-thickening phenomenon has been tied to the existence of "flow-induced macromolecular entanglements" known as hyper-entanglements which was higher at lower magnitudes of shear rates. At higher shear rate range, however, reduced resistance to flow due to these entanglements can be anticipated. Hypothetically, under conditions of high shear ($\dot{\gamma} \simeq 1000\ \mathrm{s}^{-1}$), the random coil structure of the pectin molecules are drawn to tropism, resulting in a reduction of the said hyper-entanglement, and ultimately an increasingly lower solution viscosity (Ma *et al.*, 2014).

Table 4.4. Flow (power-law) parameters for dispersions of cranberry pectin in comparison to those of commercial hydrocolloids.

Sample	Concentration ($\mathbf{wt}\%$)	Power-law model fitting parameters		
		n	*log K*	R^2
Cranberry pectin	1	0.7775	-1.6471	0.9779
	2	0.8040	-0.9154	0.9951
	4	0.7491	0.1431	0.9902
High methoxyl pectin	1	0.4366	-1.6525	0.9629
	2	0.4415	-1.2363	0.9743
	4	0.5858	-0.7507	0.9655
Wheat starch	1	1.1342	-3.4433	0.9839
	2	0.7633	-2.4017	0.9168
	4	0.8680	-2.6175	0.9384

In the case of the Newtonian plateau onset shear rate decreased with increasing concentration

(**Fig. 4.5b**), where Cross model are used to fit the rheological data of polymer solutions for values above the critical shear rate ($\dot{\gamma}_c$):

$$\eta = \eta_\infty + \frac{\eta_0 - \eta_\infty}{1+(\lambda\dot{\gamma})^m} \quad \text{Eq. 4.11}$$

where: η is the viscosity at a given shear rate $\dot{\gamma}$; η_0 and η_∞ are the asymptotic values of ZSV and infinite shear rate viscosities of cranberry pectin dispersions, respectively, and it has been observed that reliable estimation of these extrapolated parameters are best carried out over a large-enough shear rate range; λ is a time constant with dimension of time related to the structure of the fluid; while m is the dimensionless rate constant depicting the dependency of viscosity on shear rate, such that $m = 0$ indicates Newtonian fluid behaviour and $m \simeq 1$ informs increasing shear-thinning behaviour, with a value of 2/3 typifying aqueous solutions of polymers. The reciprocal of time constants, $1/\lambda$, is equated to the critical shear rate that heralds the onset of shear-thinning, i.e. $\dot{\gamma}_{c2} = 1/\lambda$ (Ma *et al.*, 2014). Such an application of Cross model was attempted on flow data for aqueous CBP solutions in the concentration range of 1.0 to 4.0 $\mathbf{wt}\%$, and the corresponding pertinent model parameters are listed in **Table 4.5**.

Table 4.5. Cross model fitting parameters of CBP solution at different concentrations ranging from 1.0 to 4.0 $\mathbf{wt}\%$.

CBP ($\mathbf{wt}\%$)	Cross model fitting parameters		
	η_∞ (Pa·s)	η_0 (Pa·s)	R^2
1.0	0.009	0.080	0.996
2.0	0.048	0.293	0.973
4.0	0.206	7.119	0.992

4.3.3.2 Effect of temperature on viscosity

The flow behaviour of 4 $\mathbf{wt}\%$ CBP solution was also examined as a function of temperature (24, 50, and 75 °C). As shown in **Figure 4.6**, a decrease in viscosity is observed as temperature increased from 24 to 75 °C for the tested CBP solution. The reason for this drop in η can be linked to thermal expansion (embodied by increasing volume) of the aqueous phase, which results in an increased intermolecular distance. Similar observation was reported for 2.5% (w/v) aqueous sodium alginate solution (Ma *et al.*, 2014).

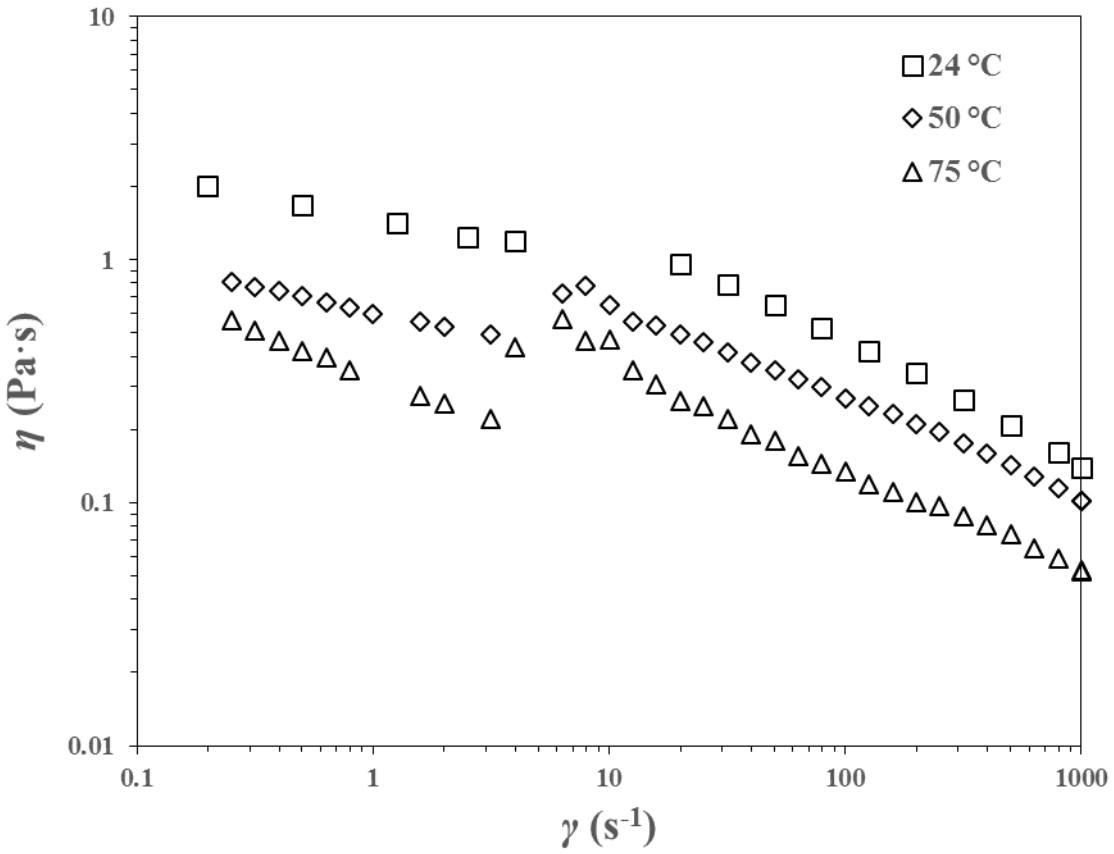


Figure 4.6. Viscosity as a function of shear rate for 4.0 **wt%** CBP solution at different temperatures (25, 50, and 75 °C).

The temperature dependency of hydrocolloids is usually investigated using the Arrhenius model:

$$\eta = A \times e^{\frac{E_a}{RT}} \qquad \text{Eq. 4.12}$$

or, when linearized:

$$\ln \eta = \ln A + \frac{E_a}{RT} \qquad \text{Eq. 4.13}$$

where η is the apparent viscosity; R is the gas constant; T, the thermodynamic temperature; A is a constant; and E_a is known as the flow activation energy, which is a measure of how much shearing energy is required by the polymer to initiate movement. Hence, the higher the magnitude of E_a, the more temperature sensitive the solution. In **Figure 4.7**, Arrhenius ($\ln \eta$ vs. $1/T$) plot for 4.0 **wt%** CBP solutions under a number of shear rate within the range of 0.5 to 1000 s^{-1}. The corresponding flow activation energies (E_a) under these conditions are reported in **Table 4.6**. E_a was obtained after performing linear regression of each plot and determining

their slope (E_a/R), which was multiplied by the ideal gas constant ($R = 8.314\ \mathrm{J\ mol^{-1}\ K^{-1}}$). The magnitudes of A are computed as the exponential of the intercept of the regression equations obtained from **Figure 4.7**. In **Table 4.6**, it is obvious that the temperature dependence of CBP aqueous solution was fairly well described by the Arrhenius equation ($R^2 \geq 0.94$).

Table 4.6. Arrhenius model parameters for temperature dependency of apparent viscosity for 4.0 **wt%** CBP solutions at different shear rates ranging from 0.5 to 1000 s^{-1}.

Shear rate $\dot{\gamma}$ (s^{-1})	0.5	1	5	10	50	100	500	1000
E_a (kJ mol^{-1})	23.49	26.29	16.91	12.89	21.50	20.96	17.33	16.40
R^2	0.99	0.99	1.00	1.00	0.99	0.99	0.95	0.94

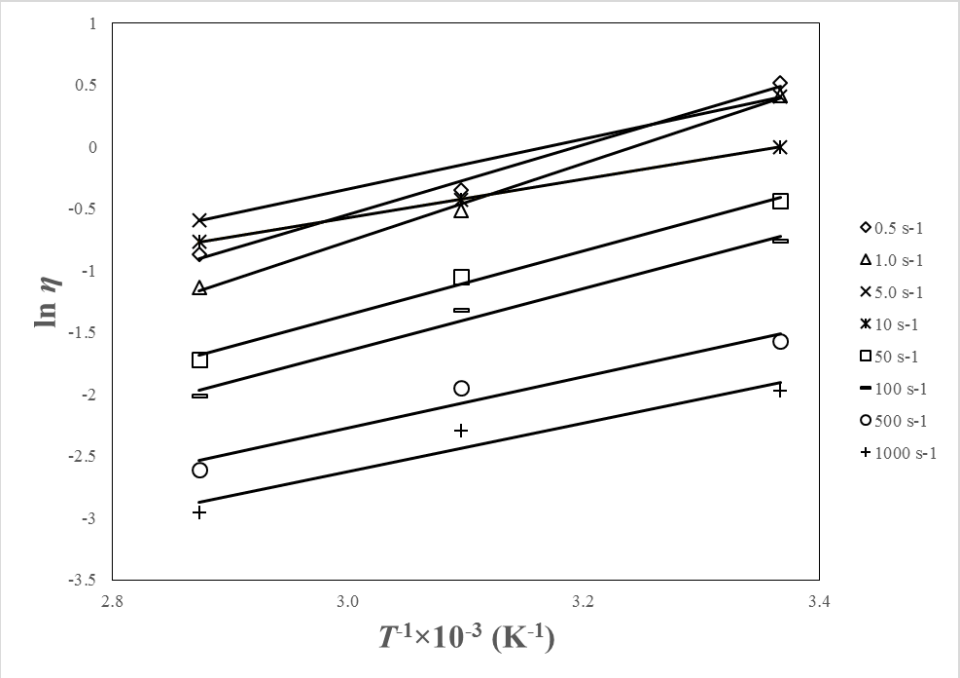


Figure 4.7. Arrhenius plots of apparent viscosity dependence on temperatures for 4.0 **wt%** CBP solution at different shear rates ranging from 0.5 to 1000 s^{-1}. Solid lines are based on fitted linearized Arrhenius equation.

The presently reported temperature-dependency for cranberry pectin correlates with the report by Marcotte *et al.* (2001), who observed high R^2 values for pectin solutions under 50 s^{-1} shear rate at different concentrations (1–3 %) in the temperature range 20 to 80 °C. Moreover, **Table 4.6** also makes it clear that E_a values are lower as shear rate increased. The implication of which is that CBP solution flowed more readily at higher shear rate. The magnitude of E_a reported herein for CBP can be considered "intermediate" (Marcotte *et al.*, 2001), thus, considerable; and this implies that aqueous solutions of CBP are sensitive to temperature under shear. In

contrast, aqueous solutions of sodium alginate and xanthan gum have been reported as being less temperature-dependent, requiring lower flow activation energies in the temperature ranges 5–35 °C and 20–80 °C, respectively (Ma *et al.*, 2014; Marcotte *et al.*, 2001).

4.3.3.3 Dynamic viscoelastic properties of monophasic pectin dispersions

While steady shear tests convey information about the viscous behaviour of fluid food systems, oscillatory (or specifically, dynamic oscillatory) tests can elicit, in addition to its viscous responses, the elastic behaviour information of the test sample. This outlook makes for the consideration of such fluids as viscoelastic systems. In principle, such viscoelastic measurements involve the application of a sinusoidal strain (or stress) of angular frequency (ω) and measuring the corresponding stress (or strain). Whereas perfectly viscous and perfectly elastic systems will exhibit a 90° and 0° phase angle (δ) between stress and strain waves, respectively, viscoelastic fluids will have a phase lag such that $0 < \delta < 90$ °C under such measurements. One of the more informative of these oscillatory tests is the frequency sweep (FS) test, and the frequency-dependent parameters obtainable by these oscillatory tests include loss (G'') and storage (G') moduli, tan δ, complex modulus ($|G^*|$), and complex viscosity ($|\eta^*|$). Importantly, FS tests should be performed within the linear viscoelastic region (LVE region or LVR) of the test material; such LVR values were ascertained for our CBP solutions by carrying out another oscillatory test, known as strain sweep test, in the strain range 0.1–100%, with results presented in **Appendix 4**. FS tests were then carried out to determine the viscoelastic behaviour of CBP solutions in the frequency range of 0.01–100 Hz (i.e., angular frequency range 0.06 to 625.2 rad s^{-1}) at 24 °C.

As **Figure 4.8** suggests, the magnitudes of G' and G'' increased with the angular frequency, which is ideal (Rao, 2014). More so, for the most part of the lower frequency, values of G'' exceeded those of G' for all cranberry pectin solutions and both moduli approached each other at higher frequencies, so that the systems exhibited sol-like behaviour in the range of frequencies studied and a sol-gel transition ($G' \approx G''$; $\delta \approx 45°$) at higher frequency. This implies that much of the input energy was dissipated through (viscous) flow, thus informing a liquid-like viscoelastic behaviour (Ma *et al.*, 2014; Rao, 2014). Under such low frequency arrangement, adjoining CBP chains are given ample time to attain a favourable conformation by slippage of the entanglement nodes. This relaxation time was instead reduced at higher frequency, making it increasingly difficult for the polymer chains to slip past one another; the entanglement nodes now act as anchor points for the chain networks. As a result, the tendency of this solution of entangled CBP to store input energy increased, with the system behaving

more like (elastic) solid (Ma *et al.*, 2014).

In addition, it is observed that values of *G*' and *G*" increased with concentration from 1.0 to 4.0 **wt%**; however, the frequencies of intersection of *G*" and *G*', known as crossover frequency (ω_c), for the solutions at different concentrations hold certain peculiarities. **Figure 4.8** shows that ω_c for CBP solutions increased with increasing concentration. This behaviour was observed for HM pectin, but is at variance with the situation for LM pectin and locust bean gum (LBG), for which crossover frequencies decreased with increasing concentrations (Lopes da Silva *et al.*, 1993).

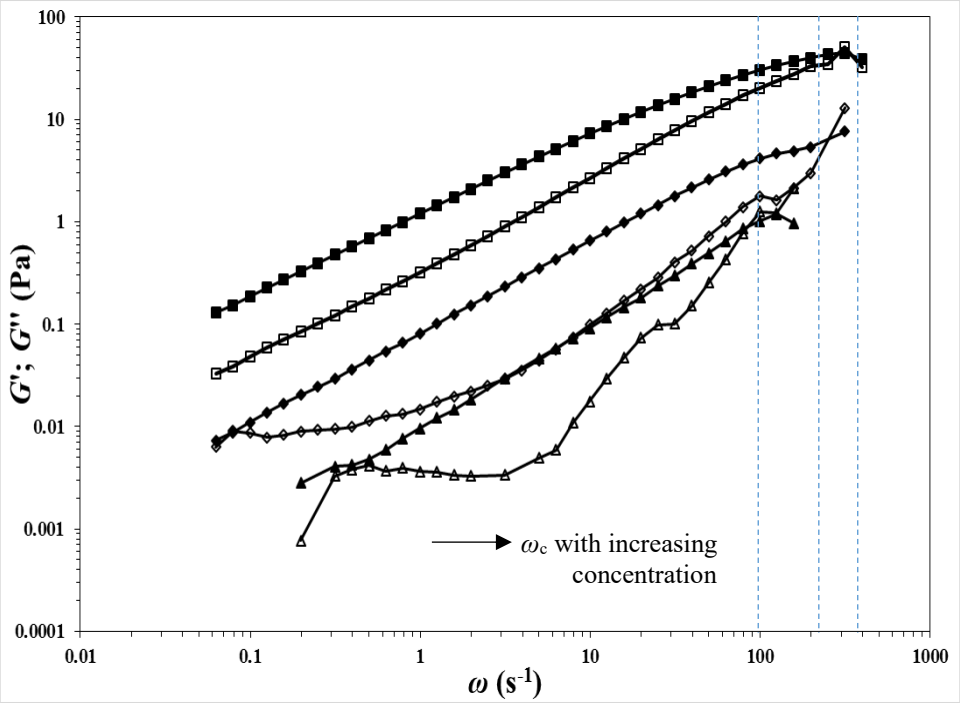


Figure 4.8. Dynamic oscillatory frequency sweep test spectra of aqueous CBP solutions of 1.0 (triangle), 2.0 (rhombus), and 4.0 **wt%** (square) concentrations, at 24 °C, showing *G*' (empty symbols) and *G*" (filled symbols) as well as their crossover frequencies (ω_c).

As for the initial 'near crossover' observed in the *G*' and *G*" plots for the solutions of 1.0 and 2.0 **wt%** concentrations at low frequency, these can be linked to the shear-thickening behaviour observed and discussed earlier for the steady shear measurement.

4.3.3.4 Correlation between dynamic and steady-shear properties— The Cox–Merz rule

Steady shear flow behaviour and linear viscoelastic functionalities (*G*', *G*", and η^*) of polymer solutions are superimposable with proven correlation (Steffe, 1996). In fact, this demonstrated relation has been proposed in the form of empirical relationships, with that of Cox and Merz

being the more appropriate (Cox and Merz, 1958):

$$\eta_a(\dot{\gamma}) = \eta^*(\omega)|_{\dot{\gamma}=\omega} \qquad \text{Eq. 4.14}$$

Such assessment bears relevance in situations where limitation(s) exist(s) in carrying out one or the other of two of the more useful rheological tests—namely, steady shear and dynamic viscoelastic measurements. With a prior knowledge of their correlation for example, steady shear flow test can then be carried out in the absence of instrumentation for oscillatory tests or even when assessing behaviour under practical applications involving higher strains beyond the linear viscoelastic region; viscoelastic behaviour can then be inferred from the flow properties. Conversely, in events where slippage and sample constituent migration (which can skew measurement) pre-empts, as in the case of some fruit juices, such limitations and characteristics can then result in dynamic rheological experiments being the method of choice, provided Cox–Merx rule holds true (Augusto *et al.*, 2011). Numerous studies on the rheological behaviour of hydrocolloid solutions and fluid food systems have shown agreement with the Cox–Merz rule.

In **Figure 4.9a–c**, such applicability or non-applicability is represented for aqueous solutions of extracted cranberry pectin. At medial shear rate/angular frequency regions (0.2–100 s^{-1} or rad s^{-1}), both rheological properties seem to coincide considerably for the 1.0 and 2.0 **wt**% concentration solutions. Conversely, at much lower and much higher shear rates/frequencies a departure from Cox-Merz rule is seen for polymer solutions at these concentrations. As for the 4.0 **wt**% solution, a clear variation is observed in the steady shear and oscillatory test parameters, again implying a departure from Cox-Merz rule. Similar departure was reported by Lopes da Silva *et al.* (1993) for 3.9% HM pectin dispersion; and like the authors, a generally lower complex viscosity than the apparent viscosity ($|\eta *| < \eta$) with convergence at higher magnitudes of shear-rate/frequency. Observed deviations from Cox–Merz rule in many studies involving biopolymer systems are attributed to system discontinuities/heterogeneities in one form or another. For instance, during the sol–gel transition in dispersions of starch (which itself is in the form of granules), the rule failed to correlate steady and dynamic shear data; the reason was that the system had become biphasic, as in an aggregation of both gelatinized and non-gelatinized starch granules (Da Silva *et al.*, 1998). As for food gum solutions, including pectin, failure of Cox–Merz to hold are ascribed to the presence of high density entanglements emanating from certain inter-polymer associations, which are enhanced by increasing concentrations (Kim and Yoo, 2006; Lopes da Silva and Rao, 2006; Morris *et al.*, 1981; Rao,

2014b).

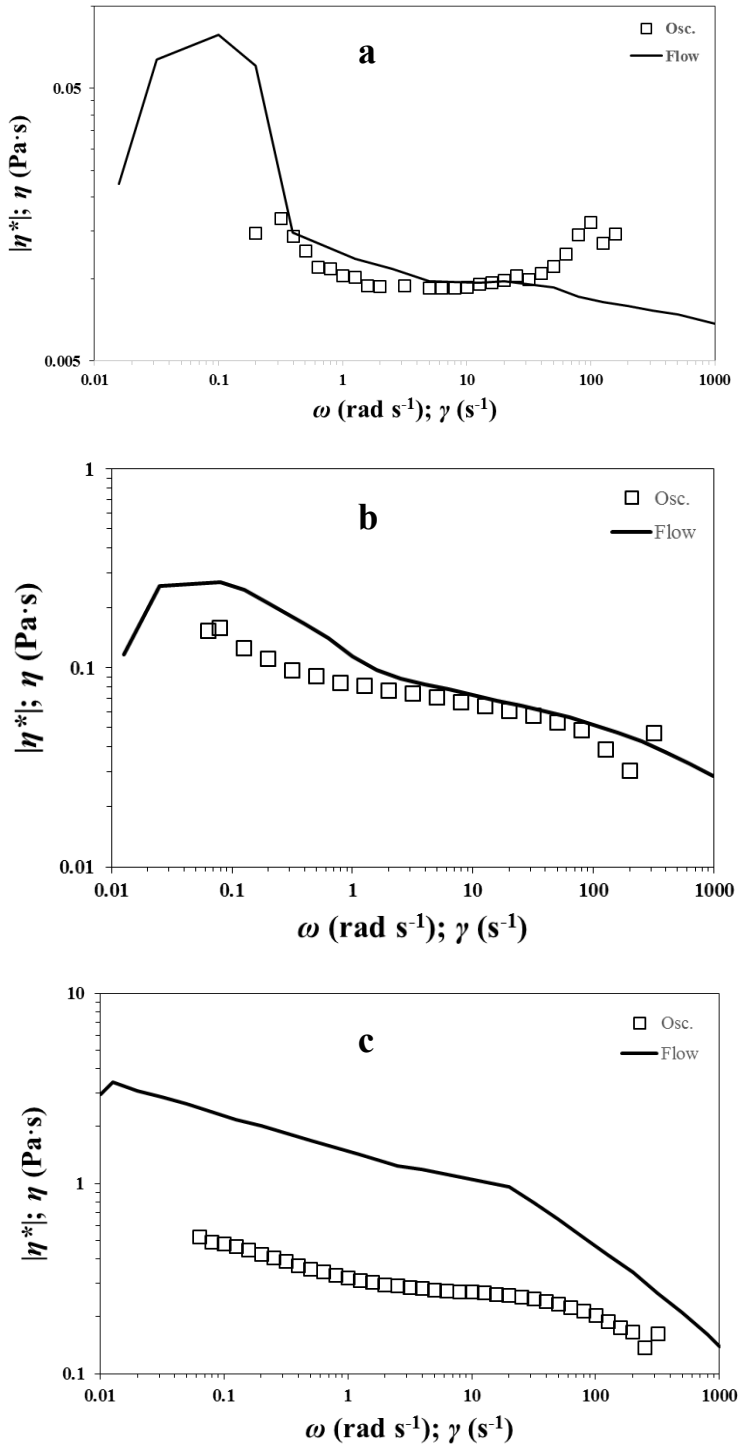


Figure 4.9. Combined (Cox–Merz) plot of complex (oscillatory) and apparent (flow) viscosities as functions of oscillatory angular frequency and shear rate, respectively, for: (a) 1.0 (b) 2.0 and (c) 4.0 **wt%** CBP solutions.

4.4 Concluding remarks

Structural and rheological characteristics of polysaccharides and their aqueous solutions do correlate with their nutritional and/or textural attributes, which in turn, informs their sensorial feel as well as consumer acceptability. Here, chemical, macromolecular and rheological studies of pectin obtained from waste cranberry (*V. macrocarpon*) pomace were undertaken. The extraction method (microwave-assisted vs. conventional heating) did influence the galacturonic acid content (43–52%) and antioxidant activity of cranberry pectin (CBP), but not the degree of esterification, such that the generally low methoxyl pectin, which was extracted by microwave heating, due to its shorter duration, exhibited higher antioxidant activity than conventionally obtained CBP. Also, Physical appearance of CBP was however darker than that of citrus pectin powder. Intrinsic viscosity of the polysaccharide was 2.15 dl g^{-1} with viscosity average molecular weight of ~39 kDa. Rheological studies on aqueous CBP solutions were then carried out considering the effects of polymer concentration (1.0–4.0 **wt%**) and temperature (24–75 °C). At all experimental concentrations, CBP solutions exhibited time-independent non-Newtonian fluid property of shear-thinning. Furthermore, their behaviours were well fitted by the Cross model. As for the temperature-dependency of their apparent viscosities, the Arrhenius model used to describe this was suitable, with flow activation energy generally decreasing with increasing shear rate, and rather high E_a values indicating a significant temperature sensitivity under shear. Small-amplitude oscillatory shear tests performed on CBP solutions revealed a fluid-like viscoelastic behaviour, whose loss and storage moduli increased with frequency and concentration, but with the former modulus leading the latter for the most part in the frequency range studied. In addition, high angular frequencies ($\omega_c \geq 100$ rad s^{-1}) are requisite to aqueous CBP solutions experiencing a sol-gel transition. Finally, monophasic dispersion of cranberry pectin did not entirely conform to the Cox–Merz rule particularly for the 4.0 **wt%** concentration. In summary, the above result allow for an understanding of the potential impart that CBP will have when used as an additive in products. Its rheological properties assessed herein also provide useful information when designing processes and processing equipment for products employing the polysaccharide.

5. SUMMARY, CONCLUSIONS AND SUGGESTION FOR FUTURE STUDIES

5.1 Summary and conclusions

Waste are often encountered as the unintended aftermath of most food processes. Despite the integral role of this industry in modern society, unsustainable waste-handling practices have resulted in stiffer environmental regulations over how its solid, liquid and gaseous wastes are being handled. The knock-on effect of increased handing cost has spurred copious enquiries not only into waste minimization (which restricts end-of-the-pipe handling methods), but also waste valorisation (in view of exploring probable new income generating sources). In view of opening new opportunities for waste utilization, one of the main themes has been the extraction of valuable compounds mainly from organic solid waste (or biomass), as embodied by the pomace from cranberry juice processing. One other challenge of today's food industry has been the ever-increasing consumer interest in reduced-fat products due to health concern. Not only do manufacturers have to reinvent their product (and perhaps process), they indeed have to do so using naturally-sourced ingredients. Biological macromolecules like polysaccharides and some proteins have been identified and even utilized as instruments for simulating the tasty fat functionality, owing to the multiplicity of rheological behaviours that can be expected from their in-solution behaviour, e.g. as fluid gels. Downstream utilizations of these biopolymers are however a result of their physicochemical properties (such as size, conformation, charge and charge distribution) as well as extrinsic factors (such as temperature, pH and ionic strength).

The mass-transfer enhancing potential of the microwave heating phenomenon and many other novel technologies (subcritical and supercritical fluids, ultrasonication, enzyme-incorporation), as heralded by improved yield and, in some cases, shorter duration, have been assessed. This study started out with the aim of obtaining functional polysaccharide from cranberry pomace—the solid residue of the juicing process—to which end a microwave-assisted extraction method was developed and optimized using the popular and reliable response surface methodology. Four factors that were assessed were temperature (40, 70, 100 °C), microwave irradiation time (2, 6, 10 min), solvent (HCl–H_20) acidity/pH (1, 2, 3), and solvent–biomass ratio (20, 30, 40 ml g^{-1}) using a Box–Behnken Design to establish their individual, synergistic and/or antagonistic influences on pectin yield (*CBP*). The quadratic model, which was generated by the method of least square, revealed that, unlike time and pH, linear terms of temperature and solvent–biomass ratio significantly influenced *CBP*. In addition, whereas there was no

significant interactions between parameters that impinged on yield, the quadratic effect of singular factor, pH, affected it. Canonical analysis revealed a saddle point rather than a clear maxima within experimental domain, thus necessitating ridge analysis for optimization, performed with statistical software SAS (v 9.4, SAS Institute Inc.). This then resulted in optimum yield under conditions: temperature, 82 °C; time, 6.08 min; solvent-biomass ratio, 22 ml g^{-1}; and pH, 1.54. *CBP* obtained at this optimum was then contrasted with the conventional heating extraction and confirmed the enhanced efficiency imparted by MW heating on the mass transfer process.

On the second note, physicochemical characteristics of recovered pectic polysaccharide were assessed. Beginning with FT-IR spectral analysis, which revealed the presence of methyl-esterified and non-methyl-esterified carboxyl groups typical of uronic polysaccharides, specifically the low methoxyl type, studies of chemical (uronic acid content, antioxidant activity), appearance (colour), macromolecular (intrinsic viscosity and molecular weight), and rheological (flow and viscoelastic) properties were undertaken, with reference made to the effect of extraction methods and conditions on such properties. Based upon findings from this study, certain uses or limitations can be conjectured for polysaccharides obtained from cranberry pomace:

1. Its antioxidant activity and relatively moderate low weight (M_v) can make it a target as an antioxidant dietary fibre.
2. Also, even though it has a low M_v, using it at higher concentration could prove useful in enhancing viscosity of food and pharmaceutical formulations.
3. The particularly low methoxyl (LM) pectin (i.e., DE<50%) obtained from cranberry can make it handy in formulations involving low sugar environment. As it is known that LM pectins gel across a broad pH range and requiring only the presence of divalent cations like Ca^{++} (which are quite abundant in food systems), these property makes CBP a potential ingredients in, for example, milk-based deserts, jams, jellies, and glazes.
4. The rather low degree of pseudoplasticity exhibited by cranberry pectin dispersion may preclude its use in practical conditions where shear-thinning behaviours are desired, e.g., during mixing or pumping.
5. It appeared that the viscoelastic behaviours of 1-2.0 **wt%** dispersion of the polysaccharide are only partly inferable from the flow data, and vice versa, in accordance with Cox–Merz rule. CBP solutions of 4.0 **wt%** concentration deviated

markedly from Cox-Merz rule.

5.2 Suggestion for future studies

1. This study involved a single response optimization of the MAE process; perhaps, preceded by factor screening design and experiment, optimizing yield along with physicochemical characteristics (i.e. multiple response optimization) will uncover more characteristics of this biopolymer.
2. More importantly, a cost-effective approach of valorising cranberry pomace, involving an integrated co-extraction process for pectin and phenolic compounds, should be undertaken. Such kinds of studies are feasible, as has been demonstrated for other biomasses like citrus fruit, mango, apple, olive, etc. Indeed, a pilot scale study will be a useful step ahead.
3. Furthermore, studies into the suitability of this novel polysaccharide in the applications proposed above should be undertaken in real food and or pharmaceutical formulations/systems; for example, its applicability as an oil-in-water emulsion stabilizer should be assessed. Another instance will be to evaluate the effect of cranberry pectin in human nutrition, as a dietary fibre.
4. Perhaps before 3 above, use of more advanced instrumentation and techniques such as HPSEC-MALLS-RI and ultracentrifugation, should be used for assessing hydrodynamic and macromolecular properties (R_g, M_w, M_n, M_z, hydrodynamic volume) of the polysaccharide. This, as well as neutral sugar assessment, can help in elucidating in-solution behaviours and help explain the science behind such behaviours.

APPENDIX A. EXTRACTION AND YIELD OPTIMISATION PROCEDURES

A.1 Extraction

Figure A.1 presents the Microwave Digestion unit used for carrying out the extraction experiments. **Figure A.2** on the other hand shows a closeup of freeze-dried pectin extracted from cranberry.

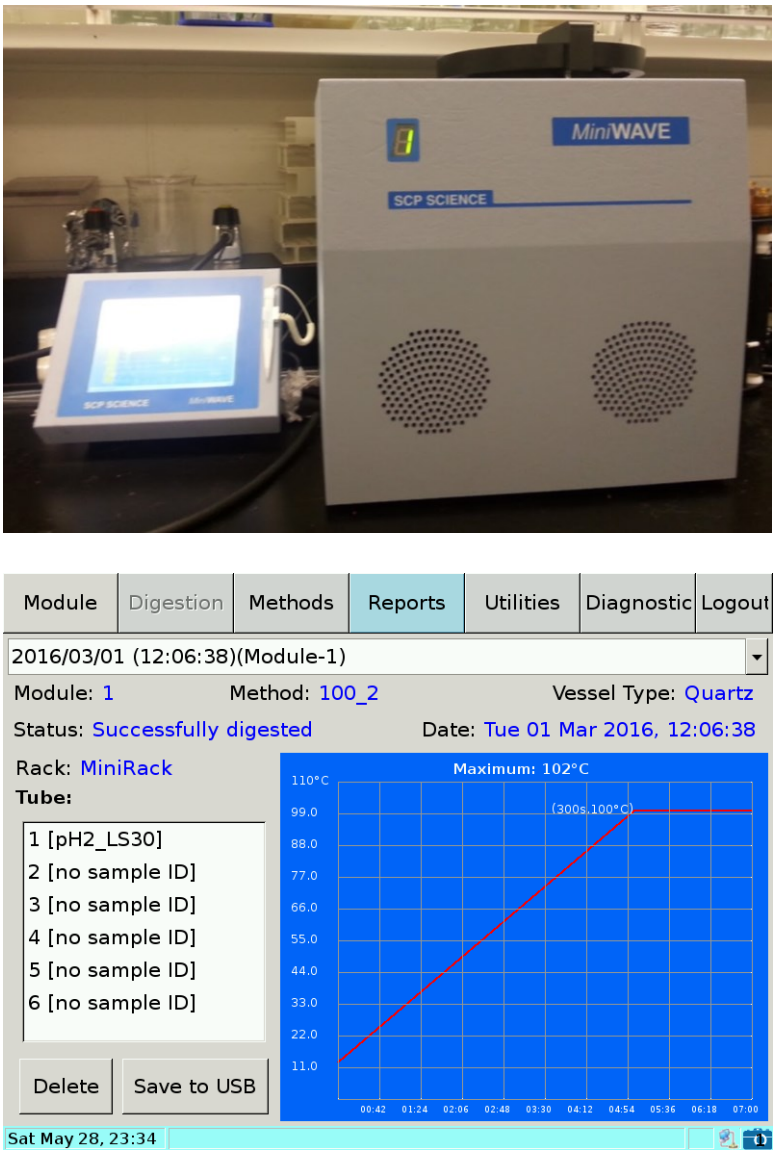


Figure A.1. MiniWAVE Microwave Digestion System (top) with a close-up of the user interface displaying real-time process conditions—temperature and time (bottom).

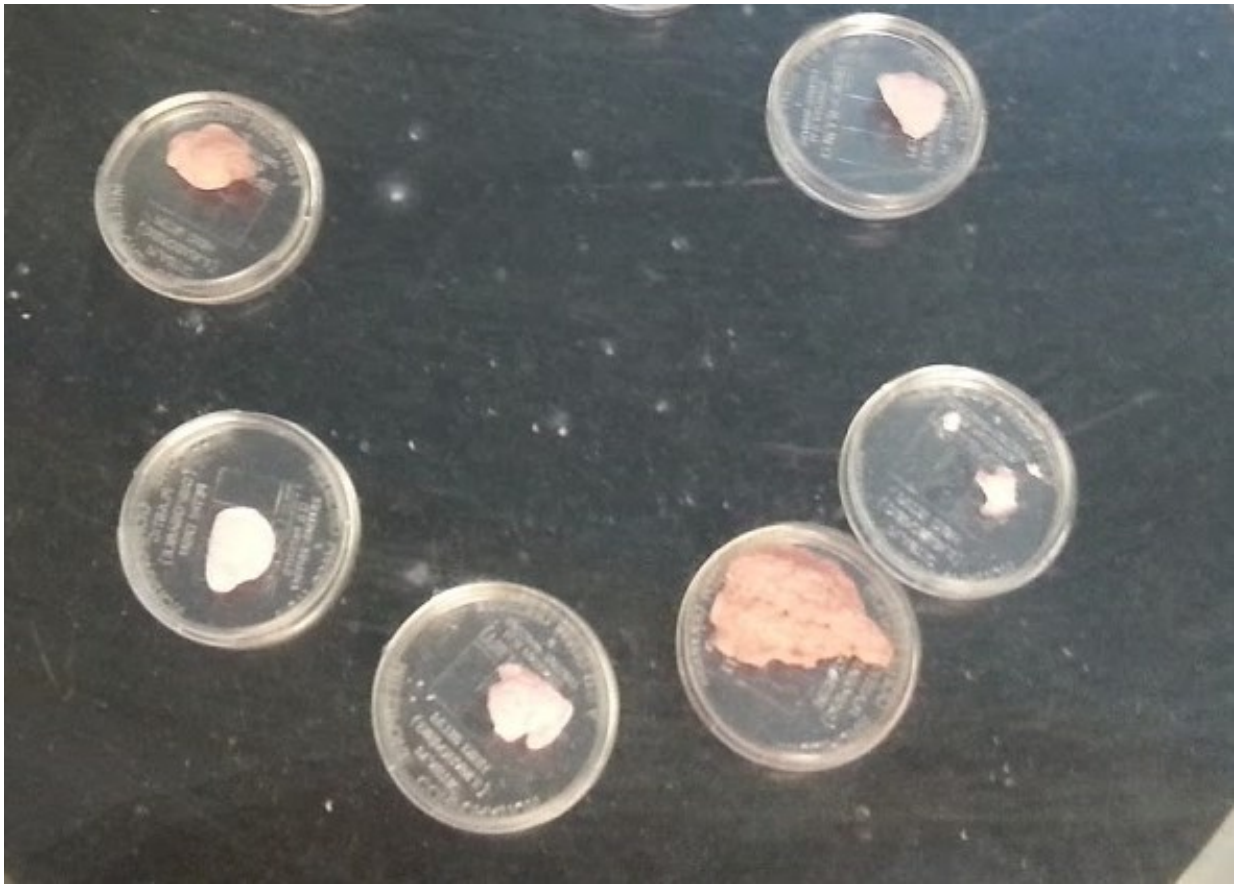


Figure A.2. Close-up of purified and freeze-dried cranberry pectin extract.

A.2 SAS codes (RSREG Procedure) for performing ridge analysis

```
data d;
   input T Time pH LS CBP;
   label T    = "Temperature (Degrees Centigrade)"
         Time = "Irradiation time (time)"
         pH   = "Solvent Acidity";
         LS   = "Solvent-Biomass Ratio (ml per gram)"
         CBP  = "Cranberry pectin yield (mg per gram)"
   datalines;
70    10    2     20    65.1
40    6     2     20    27.3
70    6     2     30    29.1
70    2     1     30    44.8
70    6     2     30    29.5
70    10    1     30    45.7
100   6     1     30    77.0
100   6     3     30    70.0
100   2     2     30    59.4
70    6     2     30    29.2
70    6     1     20    137.8
70    6     3     20    92.4
100   10    2     30    44.9
40    2     2     30    18.0
70    2     2     20    45.3
70    10    2     40    29.7
40    10    2     30    28.9
40    6     2     40    16.4
70    6     3     40    21.9
100   6     2     40    20.6
40    6     3     30    18.0
70    6     2     30    29.8
70    6     2     30    29.7
70    6     1     40    23.3
70    2     2     40    20.4
100   6     2     20    81.9
70    2     3     30    28.2
40    6     1     30    29.4
70    10    3     30    38.1
;
ods graphics on;
proc rsreg data=d plots=(ridge surface);
   model CBP=T Time pH LS / lackfit;
   ridge max;
run;
ods graphics off;
```

APPENDIX B. PHYSICOCHEMICAL CHARACTERIZATION

B.1 Galacturonic acid content determination

Ten millilitres each of standard D-galacturonic acid solutions with concentrations ranging from 100 to 1500 μM were formulated by diluting stock solutions as depicted in **Table B.1**. Absorbance readings for the standard and cranberry pectin solutions are also presented in the table, while a standard curve based on the 5 standards (A-E) is shown in **Figure B.1** and annotated with its coefficient of correlation ($R^2 = 0.9537$) and linear regression equation (y=0.0007x-0.0469).

Table B.1. The dilution series for the D-galacturonic acid samples.

Sample Label	Molar conc., (μM)	0.5 mM GalA stock solution (ml)	10 mM GalA stock solution (ml)	Volume of water (ml)	Absorbance [a], Au
A	100	2.0	-	8.0	0.079 ± 0.005
B	200	4.0	-	6.0	0.149 ± 0.015
C	500	10.0	-	0.0	0.193 ± 0.013
D	1000	-	1.0	9.0	0.565 ± 0.035
E	1500	-	1.5	8.5	1.081 ± 0.165

[a] data reported as (mean)±(standard deviation) of at least two replicates.
[b] CBP is the based on solution from MW extracted pectin from cranberry.

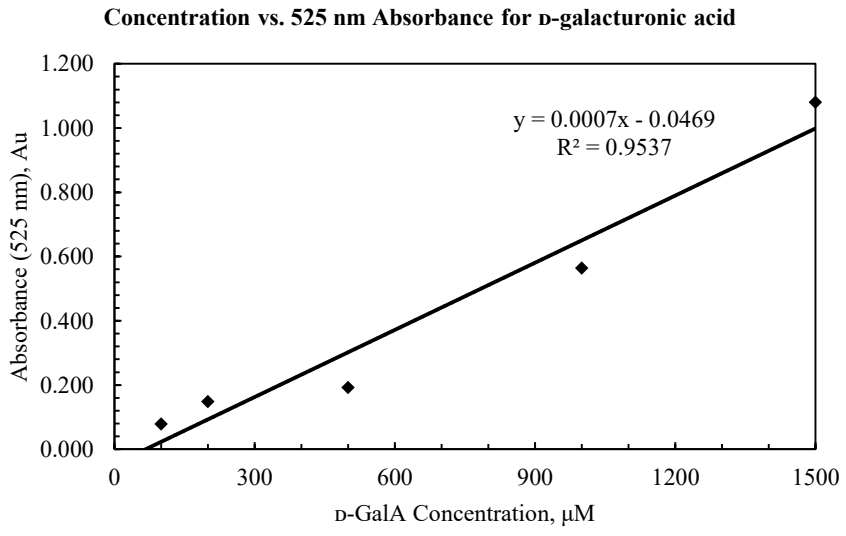


Figure B.1. Standard curve from D-galacturonic acid solutions.

B2. Linear viscoelastic (LVE) region

The LVE region of a given sample can be verified by increasing the amplitude of oscillation (stress or strain) and noting the magnitude of phase angle/lag and the amplitude ratio. Typically, the linearity limit is considered the point amplitude value at which dynamic rheological properties $|G^*|$ and $|\eta^*|$ deviate by marked amount from the constant/plateau ($\simeq$10%) (Rao, 2014). Either of creep or dynamic oscillation can be used in determining LVE region. The latter was used for CBP solutions, with a strain sweep test specifically performed. The results for the different concentrations of polymer solutions are presented in **Figure B.2**.

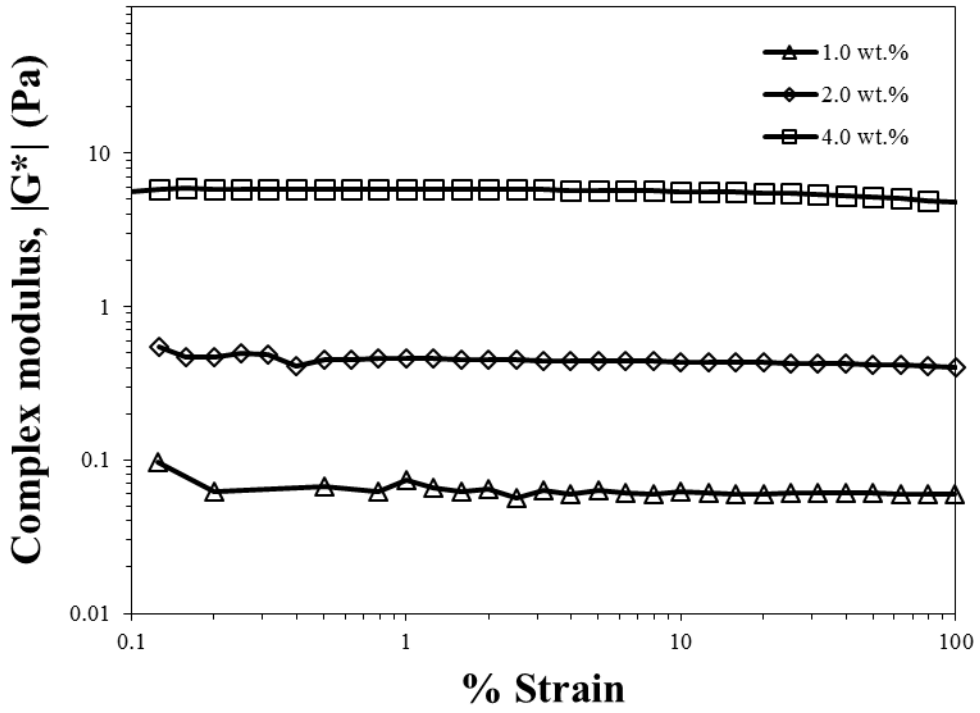


Figure B.2. Changes in complex moduli ($|G^*|$) in strain sweep of CBP solution at different concentrations ($f = 1Hz;\ T = 24\ °C$).

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