PRODUCTION OF PECTIN FROM WASTES AND LOW-GRADE PRODUCTS OF SUN-DRIED FIG PROCESSING: OPTIMIZATION OF PECTIN EXTRACTION AND CHARACTERIZATION OF ITS MAJOR PROPERTIES

A Thesis Submitted to
the Graduate School of Engineering and Sciences of
İzmir Institute of Technology
in Partial Fulfillment of the Requirements for the Degree of

DOCTOR OF PHILOSOPHY

in Food Engineering

by
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June 2023
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ACKNOWLEDGEMENTS

It is a pleasure to express my deepest thanks to my supervisor Prof. Dr. Ahmet Yemenicioğlu for his expertise, guidance, encouragement, motivation, and support throughout my thesis. I would also like to thank my thesis progress committee members Prof. Dr. Erol Şeker and Assoc. Prof. Dr. Ali Oğuz Büyükkileci for their valuable comments throughout my doctoral study. Also, I am grateful to my jury members Prof. Dr. K. Nazan Turhan and Assoc. Prof. Dr. Seda Ersus for their invaluable suggestions and comments.

I would like to express my deep gratitude to my former labmates, Derya Boyacı and Gözde Seval Sözbilen particularly for their guidance in laboratory and their friendship. I would like to thank all the researchers at IZTECH with whom I have had a series of amazing shares.

This doctoral dissertation was supported by the The Scientific and Technological Research Council of Turkey (TÜBITAK, Project no: 118 O 372). I would like to thank the Integrated Research Center at IZTECH as well as the Department of Biomedical Engineering at Yıldız Technical University for their help in some analyses.

I owe a deep sense of gratitude to my family; Begüm Esra Develi, Erkan Develi, Zeynep Özer, Elif Özer, İsmail Özer, Suna Çavdaroğlu, Nail Çavdaroğlu, Çağan Çavdaroğlu, and İrem Çavdaroğlu for their endless support and love, in all conditions. I would like to extend my heartfelt gratitude to my loving spouse, Çağrı Çavdaroğlu. I am truly fortunate to have such an incredible partner who not only shared the joys and triumphs but also stood by my side during the moments of doubt and frustration. His love, understanding, and unwavering support were the pillars that sustained me during the long hours spent researching, writing, and revising this thesis.

I dedicate this thesis to the cherished memory of my mother, Benan Koç. Her unwavering support, guidance, and inspiration continue to resonate within me. Though she is no longer with us, her impact on my academic journey and personal growth remains profound. This work is a tribute to her enduring influence.

ABSTRACT

PRODUCTION OF PECTIN FROM WASTES AND LOW-GRADE PRODUCTS OF SUN-DRIED FIG PROCESSING: OPTIMIZATION OF PECTIN EXTRACTION AND CHARACTERIZATION OF ITS MAJOR PROPERTIES

This thesis aimed extraction and characterization of pectin from processing wastes as stalks and low-grade fruits of sun-dried figs as an alternative pectin source. The extraction performed with three techniques (hot acidic, ultrasonic, enzymatic extraction) was optimized for key parameters. The hot acidic extraction, the most feasible method, yielded 11.7% crude fig stalk pectin (CSP) and 9.4% crude low-grade fig pectin (CFP) at optimal extraction conditions. The CSP had higher galacturonic acid content (GA: 34.2%) and degree of esterification (DE: 45%) than CFP (GA: 32.2% and DE: 36.7%). Purification of CSP gave pectin (PSP) with the highest GA (63%) and DE (65.9%). Despite differences in sugar compositions (D-glucose, L-rhamnose, D-galactose and L-arabinose), fig and citrus pectins displayed similar molecular weights and FT-IR profiles. The fig pectins were characterized for their gelation, antioxidant activity, water/oil absorption, emulsification/foaming capacities and stabilities, and viscosity. The properties of edible fig pectin films obtained with or without $CaCl_2$ crosslinking were also investigated. PSP films showed greater mechanical strength (15.6-19.1 MPa), but lower water vapor permeability (6.28-12.85 g.mm/m^2.day.kPa) than other films. The crosslinked CFP film exhibited the lowest solubility (32.8%) and degree of swelling. The emulsion films of CFP with eugenol (EUG) characterized and applied as a coating on whole melons effectively inhibited *Listeria innocua* (-2.2 log reduction) within 1 weeks at 10 °C. Fig pectins exhibited comparable or superior functional properties than commercial pectins, thus, utilization of low-quality figs and fig stalks into pectin could provide huge economic benefits to Turkish dried-fruit industry.

ÖZET

KURU İNCİR İŞLEME ATIKLARI VE HURDALARINDAN PEKTİN ÜRETİMİ: PEKTİN EKSTRAKSİYONUN OPTİMİZASYONU VE BAŞLICA ÖZELLİKLERİNİN KARAKTERİZASYONU

Bu tezde, alternatif bir pektin kaynağı olarak güneşte kurutulmuş incirlerin işlenmesiyle oluşan sap atıklarından ve düşük kaliteli meyvelerden pektin ekstraksiyonu ve bunun karakterizasyonu amaçlanmıştır. Ekstraksiyon, üç farklı teknik (sıcak asidik, ultrasonik, enzimatik ekstraksiyon) kullanılarak değişik koşullarda gerçekleştirilmiştir. Ekonomik olarak en uygun yöntem olan sıcak asidik ekstraksiyon ile optimum koşullarda ham incir sapı pektini için (CSP) %11,7, ham düşük kaliteli kuru incir pektini için (CFP) %9,4 verim elde edilmiştir. Galakturonik asit (GA) içeriği ve esterleşme derecesi (DE) dikkate alındığı zaman CSP (GA: %34,2, DE: %45) üretimi CFP'ye (GA: %32,2, DE: %36,7) göre daha avantajlıdır. CSP saflaştırıldığı zaman GA (%63) ve DE (%65,9) değerleri daha yüksek saf incir sapı pektini (PSP) elde edilmiştir. Şeker kompozisyonlarında (D-glikoz, L-ramnoz, D-galaktoz ve L-arabinoz) farklılıklar olmasına rağmen, incir ve turunçgil pektinleri benzer molekül ağırlığı ve FT-IR profilleri göstermiştir. İncir pektinleri, jelleşme, antioksidan aktivite, su/yağ tutma, emülsiyon/köpük oluşturma kapasite ve stabiliteleri ile viskozite özellikleri açısından karakterize edilmiştir. Ayrıca, incir pektinlerinin yenilebilir film oluşturma özellikleri, $CaCl_2$ ile çapraz bağlanmış ve bağlanmamış olarak incelenmiştir. PSP filmleri, diğer filmlere göre daha yüksek mekanik dayanıklılık (15,6-19,1 MPa) ve daha düşük su buharı geçirgenliği (6,28-12,9 g.mm/m^2.gün.kPa) gösterirken, CFP-çapraz bağlı film en düşük suda çözünürlük (%32,8) ve şişme derecesi göstermiştir. CFP kullanılarak ögenol ile oluşturulmuş ve karakterize edilmiş emülsiyon filmler tüm kavunlarda kaplama olarak uygulandığında 10 °C' de 1 hafta depolamayla *Listeria innocua*' yı önemli düzeyde (2.2 log) inaktive etmiştir. İncir pektinleri ticari pektinlere kıyasla benzer veya üstün fonksiyonel özellikler göstermiştir. Buna göre kuru incir düşük kalite meyve ve sap atıklarından katma değeri yüksek pektin üretiminin Türk kuru meyve sektörüne ekonomik katkılar sağlayabileceği açıktır.

Dedicated to my beloved mother, Benan

TABLE OF CONTENTS

LIST OF FIGURES xii

LIST OF TABLES xv

LIST OF SYMBOLS xvii

LIST OF ABBREVIATIONS xviii

CHAPTER 1. INTRODUCTION 1

CHAPTER 2. LITERATURE REVIEW 4

2.1. The Fig Fruit: Worldwide Production and Processing Wastes 4

2.2. Pectin 7

2.2.1. Pectin Structure 8

2.2.2. Pectin Sources 9

2.2.3. Pectin Extraction 16

2.2.4. Functional Properties Used in Food Applications 20

2.2.4.1. Gelation 21

2.2.4.2. Thickening 23

2.2.4.3. Emulsification 24

2.2.4.4. Edible Film and Coating Formation 25

2.2.5. Non-food Applications 27

2.2.6. Nutraceutical Potential of Pectin 28

CHAPTER 3. OPTIMIZATION OF PECTIN EXTRACTION AND CHARACTERIZATION OF MOLECULAR PROPERTIES OF FIG PECTIN 30

3.1. Introduction 30

3.2. Materials…………… 32
3.3. Methods………... 32
3.3.1. Hot Acidic Extraction 33
3.3.2. Ultrasound-assisted Extraction 35
3.3.3. Enzyme-assisted Extraction 36
3.3.4. Characterization of Molecular Properties of Fig Pectins and Commercial Pectins 36
3.3.4.1. Determination of Galacturonic Acid Content 37
3.3.4.2. Determination of Degree of Esterification............................ 38
3.3.4.3. Determination of Degree of Methylation and Acetylation by HPLC 38
3.3.4.4. Determination of Sugar Composition and Molar Ratios 39
3.3.4.5. Determination of Molecular Weight.................................... 40
3.3.4.6. Ash, Moisture and Protein Contents 40
3.3.4.7. Fourier Transform Infrared Spectroscopy Analysis 41
3.3.4.8. Color Analysis 41
3.3.5. Statistical Analysis 41
3.4. Result and Discussion 42
3.4.1. Determination of Acid Extraction Parameters 42
3.4.2. Effect of Ultrasound-assisted Extraction 46
3.4.3. Effect of Enzyme-assisted Extraction 47
3.4.4. Comparison of Yields and Pectin Properties of Different Fig Samples Extracted at Optimum Conditions 50
3.4.5. Comparison of Composition and Molecular Properties of Fig Pectins with Commercial Pectins.. 51
3.4.5.1. Molecular Weight of Fig Pectin and Commercial Pectin 56
3.4.5.2. Comparison of FT-IR Spectra of Fig Pectins and Commercial Pectins 57
3.4.5.3. Color of Fig Pectins and Commercial Pectins 58
3.5. Conclusions 60

CHAPTER 4. CHARACTERIZATION OF FUNCTIONAL PROPERTIES OF FIG PECTINS 61

4.1. Introduction ... 61
4.2. Materials.. ... 62
4.3. Methods… ... 63
4.3.1. Foaming Capacity and Foam Stability ... 63
4.3.2. Emulsifying Capacity and Emulsion Stability ... 64
4.3.3. Determination of Viscosity ... 65
4.3.4. Determination of Gelling Capacity ... 65
4.3.5. Water and Oil Absorption Capacity ... 66
4.3.6. Antioxidant Activity ... 66
4.3.7. Statistical Analysis ... 67
4.4. Result and Discussion ... 67
4.4.1. Foaming Capacity and Foam Stability ... 68
4.4.2. Emulsification Capacity, Emulsion Stability and Particle Size Characteristics ... 69
4.4.3. Viscosity ... 71
4.4.4. Gelling Capacity ... 74
4.4.5. Water and Oil Absorption Capacity ... 78
4.4.6. Antioxidant Activity ... 82
4.5. Conclusions ... 83

CHAPTER 5. DEVELOPMENT AND CHARACTERIZATION OF EDIBLE FILM FROM FIG PECTIN ... 84
5.1. Introduction ... 84
5.2. Materials.. ... 85
5.3. Methods… ... 86
5.3.1. Film Forming ... 86
5.3.2. Mechanical Tests ... 87
5.3.3. Determination of Water Vapor Permeability ... 87
5.3.4. Moisture Content and Solubility in Water ... 88
5.3.5. Swelling Degree ... 89
5.3.6. Transparency and Color of Films ... 89
5.3.7. Morphology of Film ... 90
5.3.8. Statistical Analysis ... 91

5.4. Result and Discussion 91
5.4.1. Mechanical Properties of Pectin Films 92
5.4.2. Water Vapor Barrier Properties 95
5.4.3. Solubility and Swelling of Pectin Films 98
5.4.4. Transparency and Color of Pectin Films 100
5.4.5. Morphological Properties of Films by AFM and SEM 103
5.5. Conclusions 108

CHAPTER 6. DEVELOPMENT AND CHARACTERIZATION OF EMULSION BASED EDIBLE FILM FROM FIG PECTIN 109
6.1. Introduction 109
6.2. Materials.. 111
6.3. Methods… 111
6.3.1. Acidic Extraction and Characterization of Molecular Properties of Fig Pectin 112
6.3.2. Preparation of Pectin-EUG Emulsion-Based Films and Coatings 112
6.3.3. Stability of Pectin-EUG Emulsions 113
6.3.4. Antimicrobial Activity of Films on Inoculated Agar Surface 113
6.3.5. Antimicrobial Activity of Coatings on Inoculated Webbed-Rind Melons 114
6.3.6. Mechanical Tests 115
6.3.7. Morphology of Film 115
6.3.8. Statistical Analysis 116
6.4. Result and Discussion 116
6.4.1. Stability of Pectin-EUG Emulsions 116
6.4.2. Antilisterial Activity of Films in Zone Inhibition Test 121
6.4.3. Antilisterial Activity of Films at Inoculated Webbed Melon Surfaces 123
6.4.4. Mechanical Properties of Films 126
6.4.5. Morphology of Pectin Films 127
6.5. Conclusions 129

CHAPTER 7. SUMMARY AND CONCLUSIONS 130

REFERENCES 133

APPENDICES 164
APPENDIX A. STANDARD CURVES USED IN THE ANALYSES 164
APPENDIX B. CORRELATION TABLES 169

VITA……………………………………………………………………………..171

LIST OF FIGURES

Figure **Page**

Figure 2.1. Photographic images of samples of different types of figs [fig puree waste (A), dried fig stalk (B), low-quality dried figs (C)]. 5

Figure 2.2. Three-dimensional view of polymer arrangement in the plant cell wall. Created with BioRender.com based on information in Cui et al. (2021) with permission from Elsevier. 8

Figure 2.3. Schematic representation of the structure of pectin. Created with BioRender.com based on information in Reichembach and Petkowicz (2021) with permission from Elsevier. 10

Figure 3.1. Flow diagram of pectin production. 34

Figure 3.2. Purification of pectin after acid extraction. 35

Figure 3.3. FT-IR spectra of commercial pectins and different fig pectins. 58

Figure 3.4. Photographic images of commercial pectins and different fig pectins: [citrus pectin (CP) (A), apple pectin (AP) (B), crude low-grade dried fig pectin (CFP) (C), crude fig stalk pectin (CSP) (D) and purified fig stalk pectin (PSP) (E)]. 59

Figure 4.1. Foam capacity (A) and foam stability (B) of commercial citrus and different fig pectins. Each data point is shown as the average of three replicates. The error bar indicates standard deviation. a-c and A-B at each bar denote a statistically significant difference separately ($p \leq 0.05$). 69

Figure 4.2. Emulsification capacity (A), emulsion stability (1st Day of emulsion stability (B); 7th Day of emulsion stability (C)) and droplet sizes (D) of commercial and different fig pectin emulsions at different concentrations (1-3%, w/v) during room temperature storage at 25 °C. Each data point is shown as the average of three replicates. The error bar indicates standard deviation. Different letters within experiment represent significant difference at $p \leq 0.05$. 72

Figure **Page**

Figure 4.3. Flow behavior of 3% (w/v) solutions of commercial pectins and different fig pectins at 23 °C. 73
Figure 4.4. Viscosity values of commercial pectins and different fig pectins at 3% (w/v) concentration. Each data point is shown as the average of three replicates. The error bar indicates standard deviation. Different letters represent significant difference at $p \leq 0.05$. 73
Figure 4.5. The least gelling concentration of CFP gels. The pectin concentration (%, w/w) decreased from left to right as follows: 1.2, 1, 0.8, 0.6 and 0.4%. 75
Figure 4.6. Photographic images of CP (A), CSP (B) and CFP (C) gels. 75
Figure 5.1. Water solubility (dark grey) and moisture (light grey) of commercial pectin and different fig pectin films. Each data point is shown as the average of three replicates. The error bar indicates standard deviation. a-d and A-D at each bar denote a statistically significant difference separately ($p \leq 0.05$). -Ca++: denoted for crosslinks for all pectins. 99
Figure 5.2. The swelling curves of commercial pectin and different fig pectin films. Each data point is shown as the average of three replicates. The error bar indicates standard deviation. -Ca++: denoted for crosslinks for all pectins. 100
Figure 5.3. Photographic images of commercial pectin and different fig pectin films. The control films; A) CP, B) AP, C) CFP, D) CSP, E) PSP and the crosslinked films; F) CP-Ca^{++}, G) AP-Ca^{++}, H) CFP-Ca^{++}, I) CSP-Ca^{++}, J) PSP-Ca^{++}. 102
Figure 5.4. The surface morphologies of commercial pectin and different fig pectin films: (A) CP; (B) AP; (C) CFP; (D) CSP; E) PSP; (F) CP-Ca^{++}; (G) AP-Ca^{++}; (H) CFP-Ca^{++}; (I) CSP-Ca^{++}; and J) PSP-Ca^{++}. 106
Figure 5.5. The topographic images of commercial pectin and different fig pectin films: (A) CP; (B) AP; (C) CFP; (D) CSP; E) PSP; (F) CP-Ca^{++}; (G) AP-Ca^{++}; (H) CFP-Ca^{++}; (I) CSP-Ca^{++}; and J) PSP-Ca^{++}. 106
Figure 5.6. The surface morphology of commercial pectin and different fig pectin films: (A) CP; (B) AP; (C) CFP; (D) CSP; E) PSP; (F) CP-Ca^{++}; (G) AP-Ca^{++}; (H) CFP-Ca^{++}; (I) CSP-Ca^{++}; and J) PSP-Ca^{++}. (Magnification: 500×, Scale bar: 300μm). 107

Figure **Page**

Figure 5.7. The cross-sectional morphology of commercial pectin and different fig pectin films: (A) CP; (B) AP; (C) CFP; (D) CSP; E) PSP; (F) CP-Ca^{++}; (G) AP-Ca^{++}; (H) CFP-Ca^{++}; (I) CSP-Ca^{++}; and J) PSP-Ca^{++}. (Magnification: 2500×, Scale bar: 50μm).. 107

Figure 6.1. Photographic images of pectin-eugenol emulsions; (A) CFP-EUG (0.25%, w/w), (B) CFP-EUG (1 %, w/w), (C) CFP-EUG (2%, w/w), (D) CP-EUG (0.25%, w/w), (E) CP-EUG (1%, w/w), (F) CP-EUG (2%, w/w).. 118

Figure 6.2. Zone inhibition photos of (A) CFP control, (B) CFP-EUG (0.25%, w/w) , (C) CFP-EUG (0.5%, w/w), (D) CFP-EUG (1%, w/w), (E) CFP-EUG (2%, w/w) on *L. innocua*.. 122

Figure 6.3 Zone inhibition photos of (A) CP control, (B) CP-EUG (0.5%, w/w), (C) CP-EUG (1%, w/w), (D) CP-EUG (2%, w/w) on *L. innocua*............ 122

Figure 6.4. Zone-inhibition based antimicrobial activity of CFP-EUG and CP-EUG films on *L. innocua*. Each data point is shown as the average of two replicates. The error bar indicates standard deviation. Data with different letters are significantly different ($p \leq 0.05$). 123

Figure 6.5. Photographic images of (A) uncoated, (B) CP-EUG (2%, w/w) coated, (C) CFP-EUG (2%, w/w) coated Galia melons. 125

Figure 6.6. Antimicrobial effects of CFP-EUG and CP-EUG coatings with 2% (w/w) EUG on *L. innocua* inoculated onto whole Galia melons during cold storage at 10 °C. Each data point is shown as the average of two replicates. The error bar indicates standard deviation. Data with different letters are significantly different ($p \leq 0.05$). 126

Figure 6.7. Photographic images of pectin films: (A) CFP; (B) CP; (C) CFP- EUG (2%, w/w); (D) CP-EUG (2%, w/w)... 127

Figure 6.8. Effect of 2% (w/w) EUG on the cross-sectional morphology of pectin films: (A) CFP; (B) CP; (C) CFP-EUG; (D) CP-EUG (Magnification: 10,000×)... 129

LIST OF TABLES

Table **Page**

Table 2.1. Nutrient content of 100 grams of fresh and dried figs (Aksoy et al 2001). 7

Table 2.2. Structural characteristics and functional properties of pectins extracted from various sources with different methods. 11

Table 3.1. Yield, galacturonic acid content and degree of esterification of pectins obtained from fig puree at different process conditions. 45

Table 3.2. Yield, galacturonic acid content and degree of esterification of pectins obtained from low grade dried fig using ultrasound-assisted extraction. 47

Table 3.3. Yield, galacturonic acid content and degree of esterification of pectins obtained from low grade dried fig using enzyme-assisted extraction.......... 49

Table 3.4. Yield, galacturonic acid content and degree of esterification of pectins obtained from low grade dried fig using different extraction methods. 50

Table 3.5. Yield, galacturonic acid content and degree of esterification of pectins obtained from different fig wastes. 51

Table 3.6. Different characteristics of commercial pectins and different fig pectins. 55

Table 3.7. Comparison of molecular weights of commercial citrus pectin and crude fig stalk pectin. 57

Table 3.8. Color values of commercial pectins and different fig pectins. 59

Table 4.1. Functional properties of commercial pectins and different fig pectins. 79

Table 4.2. Texture profile analysis of pectin gels at different concentrations. 80

Table 5.1. Mechanical properties of commercial pectin and different fig pectin films. 94

Table 5.2. Correlations between mechanical and molecular properties of commercial pectin and different fig pectin films. 95

Table 5.3. The water vapor permeability properties of commercial pectin and different fig pectin films. 97

Table 5.4. Correlations between water vapor permeability and molecular properties of commercial pectin and different fig pectin films.................................... 98

Table **Page**

Table 5.5. Transparency (T_{600}) and color values (L*, a* and b*) of commercial pectin and different fig pectin films. 103

Table 5.6. Morphological parameters of commercial pectin and different fig pectin films from AFM analysis. 105

Table 6.1. Turbidity values of CFP-EUG and CP-EUG emulsions during cold storage at 10 °C. 119

Table 6.2. Droplet sizes of CFP-EUG and CP-EUG emulsions during cold storage at 10 °C. 120

Table 6.3. Mechanical properties of CFP and CP films at different EUG concentrations. 128

LIST OF SYMBOLS

%	Percent
×g	G-force
°C	Celcius degree
μL	Microliter
μm	Micrometer
Da	Dalton
g	Gram
kcal	Kilocalories
kDa	Kilodalton
kHz	Kilo hertz
kPa	Kilopascal
m	Meter
mA	Milliamper
min	Minutes
mL	Milliliters
mm	Millimeter
mM	Milli molar
MPa	Megapascal
mPa.s	Millipascal second
mPas	Millipascal second
N	Newton
p	Probability
ϕ	Flux per stator pole
Pa	Pascal
pKa	Acid dissociation constant
r	Pearson’s coefficient of correlations
R2	The coefficient of determination
R_{max}	Maximum verticle distance between the highest and lowest points
rpm	Round per minute
R_{rms}	Surface roughness
s	Second
v/v	Volume per volume
W	Watt
w/v	Weight per volume
w/w	Weight per weight

LIST OF ABBREVIATIONS

a*	Redness value in color analysis
ABTS	2,20-azino-bis(3- ethylbenz-thiazoline-6-sulfonic acid)
AFM	Atomic force microscopy
amp	Amplitude
ANOVA	Analysis of variance
AOAC	Association of Official Agricultural Chemists
AP	Apple pectin
ASTM	American Society for Testing and Materials
AUC	Area under the curve
b*	Yellowness value in color analysis
BSA	Bovine serum albumin
CA	Citric acid
Ca^{++}	$CaCl_2$ crosslinking
CFP	Crude low-grade dried fig pectin
CP	Citrus pectin
CSP	Crude fig stalk pectin
D[3,2]	The surface mean diameter of particle size
DA	Degree of acetylation
DE	Degree of esterification
D-Gal	Galactose
D-Glc	D-glucose
DLD1	Colorectal adenocarcinoma cell line isolated from the large intestine
DLS	Dynamic light scattering
DM	Degree of methylation
EAB	Elongation at break
EAE	Enzyme-assisted extraction
EC	Emulsification capacity
ELV	Emulsified layer volume
ES	Emulsion stability
EU	European union
EUG	Eugenol
FC	Foaming capacity
FS_{180min}	Foam stability at 180th minutes
FS_{30min}	Foam stability at 30th minutes
FT-IR	Fourier transform infrared spectroscopy
GA	Galacturonic acid
GAE	Gallic acid equivalent

GAGO20	Glucose enzyme kit
GRAS	Generally recognized as safe
HAE	Hot acid extraction
HG	Homogalacturonan
HMP	High-methoxyl pectin
HPGPC	High-performance gel permeation chromatography
HPLC	High performance liquid chromatography
HT-29	Human colorectal adenocarcinoma cell line
HuCC	Human cholangiocellular carcinoma cell line
HWE	Hot water extraction
K562	Human leukemia cell line
K-ARGA	Arabinose and galactose enzyme kit
KBr	Potassium bromide
K-RHAMNOSE	Rhamnose enzyme kit
L*	Luminous value in color analysis
L-Ara	Arabinose
LDPE	Low-density polyethylene
LGC	Least gelling concentration
LMP	Low-methoxyl pectin
L-Rha	Rhamnose
MAE	Microwave-assisted extraction
MEFE	Moderate electric field extraction
MemE	Membrane extraction
Mn	Number weighted molecular weight
MW	Molecular weight
Mw	Weight average molecular weight
Mw/Mn	Polydispersity index
n.d.	Not determined
NTU	Nephelometric turbidity units
OAC	Oil absorption capacity
OHAE	Ohmic heating-assisted extraction
PSP	Purified fig stalk pectin
R-1	Sugar molar ratio representing the linearity of pectin
R-2	Sugar molar ratio representing RG-I fraction of pectin
R-3	Sugar molar ratio representing the degree of branching of RG-I
R-4	Sugar molar ratio representing the length of galactose branching in RG-I
RG-I	Rhamnogalacturonan I
RG-II	Rhamnogalacturonan II
RH	Relative humidity
RID	Refractive index detector
SEM	Scanning electron microscopy
SLR	Solid-liquid ratio
SW	Swelling degree

SWE	Subcritical water extraction
T600	Transparency
TEAC	The Trolox equivalent antioxidant capacity
Temp	Temperature
TPA	Texture profile analysis
TPC	Total phenolic content
Trolox	6-hydroxy-2,5,7,8-tetra- methylchroman-2-carboxylic acid
TS	Tensile strength at break
UAE	Ultrasound-assisted extraction
UEAE	Ultrasound- and enzyme-assisted extraction
UMAE	Ultrasound- and microwave-assisted extraction
UV	Ultraviolet
WAC	Water absorption capacity
Wv	Initial volume of emulsion
WVP	Water vapor permeability
YM	Young's modulus

CHAPTER 1

INTRODUCTION

Ficus carica L., also known as the common fig, is a cultivated fig in the family Moraceae, which has been grown extensively in the Middle East and Mediterranean region. The genus Ficus is an important and ancient source of food and medicine in human culture, with numerous species ranging from 600 to 1900 (Stover et al. 2007). Turkey is the largest producer and exporter of dried figs which met approximately 53% of the world fig export, with dried figs and fig paste being the primary export products and provided over 246 million dollars of income in the 2021/22 season (Anonymous 2022). The sun-drying applied to figs is a very old process dating back to ancient times. Drying figs starts up to a certain moisture content on the tree and continues after falling to the ground, then finally ends up drying under the sun on mats (classical method) or in prefabricated greenhouses (modern method) performed by farmers. The effect of sun-drying process on the product quality depends on the temperature and humidity values before and during the harvest season. Also, the highly variable agricultural practices applied to the figs throughout the season have effects on quality. Therefore, the resulting products are quite heterogeneous in terms of quality. There are three different fig qualities: extra quality, class I, and class II (UNECE 2016). Generally, figs come to the facility between 18-22% moisture and then are taken to cold storage until they are fumigated, washed, and sorted for mold and aflatoxins. After classification, a considerable amount of fruit is separated as sub-standard, often showing defects such as insect bites, mold, sunburn, splitting, crushing, and excessive drying and hardening. These fruits are usually processed into molasses or alcohol after removing mold and aflatoxins. In contrast, some of the ones in good condition are sometimes processed into Turkish delight and jam.

Until a decade ago, a large portion of the dried figs exported from our country was exported in bulk and large quantities with low humidity (at 18-22%). However, nowadays, since the demand of soft dried figs is increasing, they are rehydrated to a medium humidity level (at 35-38%). This way, the medium-humidity fruits are packaged in small or medium-sized portions to pasteurize by heating or microwave and exported as ready for consumption. In this method, fig stalks are removed beforehand and often discarded as waste due to their rigid structures. According to the experience of laborers who perform this process, the cut stalks contain part of the flesh tissue that changes between 1 and 1.5% of total fruit weight. The significant quantity of waste produced by the end of each season results in the loss of valuable resources and increases both environmental and economic concerns. Therefore, in recent years, companies have requested opportunities to utilize the separated stalks. Rather than landfill disposal or utilization as animal feed, these wastes can be used to extract value-added products such as pectin.

As it is known, pectin is widely used not only in the food industry but also in the biomedical, pharmaceutical, and cosmetic industries due to its technological and functional properties as well as its positive effects on health (Gilani et al. 2008; Rezvanain et al. 2017; Yang et al. 2015; Noreen et al. 2017; Muñoz-Almagro, Montilla, and Villamiel 2021). Pectin is a complex heteropolysaccharide found in the cell wall of plants consisting mainly of α-(1,4)-linked D-galacturonic acid backbone with different degrees of esterification. Pectin is extracted from plant tissues by three different methods: chemical, physical, or enzymatic. Both mineral acids such as sulfuric, hydrochloric, phosphoric, and nitric acid and organic acids such as citric, tartaric, lactic, acetic, and oxalic acid are used for pectin extraction (Ma et al. 2013). To increase the extraction yield, many innovative technologies such as ultrasound, microwave, and enzymes such as cellulases are used in the literature (Bagherian et al. 2011; Yuliarti, Goh, Matia-merino, et al. 2015). Commercially, pectin is produced from citrus and apple processing wastes by chemical extraction based on acid hydrolysis at high temperatures (Yuliarti, Goh, Matia-merino, et al. 2015). There have been a vast number of studies that are being carried out on pectin production from various sources as an alternative to citrus pectin to meet the increasing demands of the global pectin market (Cui et al. 2021; Ciriminna et al. 2016; Khedmat et al. 2020; Liu et al. 2020). While sunflower stalks, a byproduct of the oil industry, and sugar beet pulp, a byproduct of the sugar industry, have been identified as prominent pectin sources, other wastes from fruit processing such as tomato, carrot,

pumpkin, and peels from fruits such as passion fruit, watermelon, and banana are also being investigated as potential pectin sources (Dranca and Oroian 2018). Since the molecular, functional, and health effects of pectins from different sources are quite variable, investigating the properties of pectins obtained from alternative sources, particularly from plant waste, has become a very popular topic (Reichembach and Petkowicz 2021).

Sun-dried figs are known for their various positive effects on health, mainly gastrointestinal functions. These health effects are primarily attributed to their content of soluble dietary fiber, pectin, and polyphenols (Trad, Ginies, et al. 2014a). One of the essential health benefits of dried figs is their laxative effect, mainly due to the soluble pectin content, making this ancient fruit a functional food (Simmons and Preedy 2016; Rtibi et al. 2018). Therefore, extracting pectin from figs and using the obtained pectin in alternative foods and functional food production is a unique research topic. Indeed, Chen et al. (2015) extracted polysaccharides from dried figs using complex enzymes such as pectinase, papin and cellulose with different concentrations and investigated their monosaccharide composition and molecular composition weights as well as immunological activities on both animals and cells. In 2018, another study related to the extraction and characterization of polysaccharides from *Ficus carica* based on immunological assays was published by Du et al. (2018). Fig pectin is not directly mentioned in these studies. Recently, Gharibzahedi, Smith & Guo (2019a; 2019b) produced pectin from the peel of fresh Iranian figs and characterized its molecular properties, some functional properties, and health effects such as antioxidant and anticancer effects. However, to the best of our knowledge, no study has investigated the qualitative and quantitative characteristics of the extracted pectin from fig processing wastes and low-grade products. In this study, for the first time in the literature, the optimization of pectin production from wastes and low-grade products of sun-dried fig processing was investigated, and the molecular and functional properties of fig pectin were characterized. In addition, this study shows the effects of ultrasound and enzyme on the yield and quality of extracted pectin with the conventional method.

CHAPTER 2

LITERATURE REVIEW

2.1. The Fig Fruit: Worldwide Production and Processing Wastes

Fig is one of the most valued edible fruits in the commercial market or export products of the world. The *Ficus carica* L., also known as common fig, is the one of the members of family Moraceae originated in the Middle East and produced nowadays in countries that have a Mediterranean climate, such as California, South America, Turkey, Iran, Spain, Afghanistan and Greece (Barolo, Ruiz Mostacero, and López 2014; Anonymous 2021; İrget et al. 2008). Due to the climatic and ecological demands of figs, a few countries can produce. In the western Aegean Region of Turkey, specifically in the Small and Big Meander valleys around Aydın and İzmir provinces has been cultivated the 65% of fig trees. The main variety found in this region is known as Sarılop (syn. 'Calimyrna'). This cultivar is very suitable for sun-drying process that almost all fruits grown in the Meander Basin are processed for sun-drying and exported (İrget et al. 2008; Aksoy et al. 2001). Their sugar level, size, taste, texture, light color and soft gum structure are the most suitable for drying under the sun (Simmons and Preedy 2016). Turkey is the world's leading producer of dried fig producer with 53% of the total production accounted for 75,000 metric tons of product in 2021/2022 season (Anonymous 2022). 30% of the fig produced are consumed as fresh fruit in the domestic market, 70% as sun-dried figs in the foreign and domestic market (Anonymous 2018). The dried fig exports are not only dried fruits, but some of them are paste, liquid paste, diced and cubic shaped products and

scrap. Especially, fig paste (sometimes with added wheat and corn flour, whey, syrup, oils, and other ingredients) is used in biscuit production and bakery products, while cube shaped cut products are consumed by mixing them with cereals and other fruits. Low-grade fruits are mainly used in the production of molasses and ethyl alcohol can be produced from scrap figs. During ethanol production, fig seeds come out and can be utilized in the paint, cosmetics and pharmaceutical industries (Anonymous 2020).

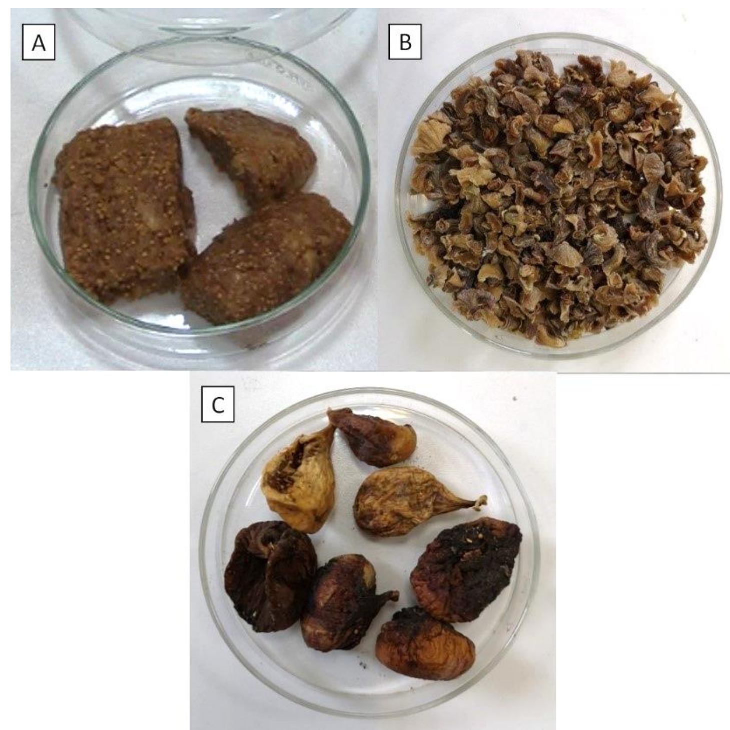

Figure 2.1. Photographic images of samples of different types of figs [fig puree waste (A), dried fig stalk (B), low-quality dried figs (C)].

Dried figs have a special place among dried fruits with their high calorie values, mineral and nutritional contents. Table 2.1 is shown the nutritional value of both fresh and dried figs. Thus, it is evident that dried figs are a rich source of energy, minerals, and fiber. Also, it is rich in essential amino acids, phenolic and flavonoid compounds, in

addition to polysaccharides, anthocyanins, phytosterols, and fatty acids (Doymaz 2005; Çalişkan and Aytekin Polat 2011; Kelebek et al. 2018; Solomon et al. 2006).

Fresh figs are susceptible to microbiological spoilage even in cold weather conditions. Therefore, the oldest method sun-drying has long been applied to the fresh figs to increase its durability (Doymaz 2005). Harvest of dried figs is achieved by collecting the fruits that fall on the soil surface by hand. These figs have already started to dry on the tree and are shrinking. After that they are put in plastic crates or lying on mats to further drying and reaching the equilibrium humidity from 30-50% up to 20-22% moisture content. During the production and processing of dried figs, a significant amount of low-grade products and wastes are produced. Based on the UNECE (2016) standard for quality control of dried figs, there are 11 items in the minimum requirements for the dried figs under the three main categories as “Extra” Class, Class I and Class II. The world estimated consumption of dried figs increase by 1.19-fold from 135,000 metric tons to 160,650 metric tons between 2018-2019 (Anonymous 2022; 2021). Thus, the demand for different type of fig products in the world market causes the increase of wastes during the processing of the fig. One of the examples of this demanding dried fig product is the consumption of ready to eat portion-pack figs (medium moisture at 35-38%). During their production, the stalks of the figs are cut, and some part of fruit is discarded together with the stalk (1 and 1.5% of total fruit weight) as waste and treated as animal feed. It is stated that the annual dried fig processing capacity of fig processing companies is between 100-5500 tons (Çobanoğlu 2012). Thus, it is estimated that 100-150 tons of stalk waste per year can be produced. These products have 2-3 times less price than sun-dried fig due to quality differences. In order to prevent loss of nutrients and economical value with these wastes, they can be valorized using greener technologies to produce highly functional products as pectin.

Table 2.1. Nutrient content of 100 grams of fresh and dried figs (Aksoy et al 2001).

Nutritional Value	Fresh Fig	Dried Fig
Water (%)	84.6	16.8
Protein (%)	1.3	3.6
Fat (%)	0.3	1.6
Carbohydrate (%)	9.5	52.9
Energy (kcal)	45	300
Total sugar (%)	9.5	52.9
Glucose (%)	5.2	28.6
Fructose (%)	4.1	22.7
Sucrose (%)	0.3	1.6
Fibre (%)	2.3	12.4
Caroten (µg)	150	64
Vitamin B1 (mg)	0.03	0.08
Vitamin B6 (mg)	0.08	0.26
Vitamin C (mg)	2	1
Potassium (mg)	200	970
Calcium (mg)	38	250
Magnesium (mg)	15	80
Phosphorus (mg)	15	89
Iron (mg)	0.3	4.2
Zinc (mg)	0.3	0.7

2.2. Pectin

Pectin is one of the most important biopolymers produced from the byproducts of vegetable and fruit industry. In the later sections, its structure, sources, extraction methods and functional properties are discussed in detail.

2.2.1. Pectin Structure

Pectin is a group of heteropolysaccharide formed by the binding of D-galacturonic acid units with α-(1 → 4) glycosidic bonds which found in primary cell wall and intercellular regions of the higher plants along with other cell wall components such as hemicellulose and cellulose as shown in Figure 2.2. (Taboada et al. 2010). It acts as a cement material in plant cell wall by providing integrity and strength through contributing intercellular adhesion.

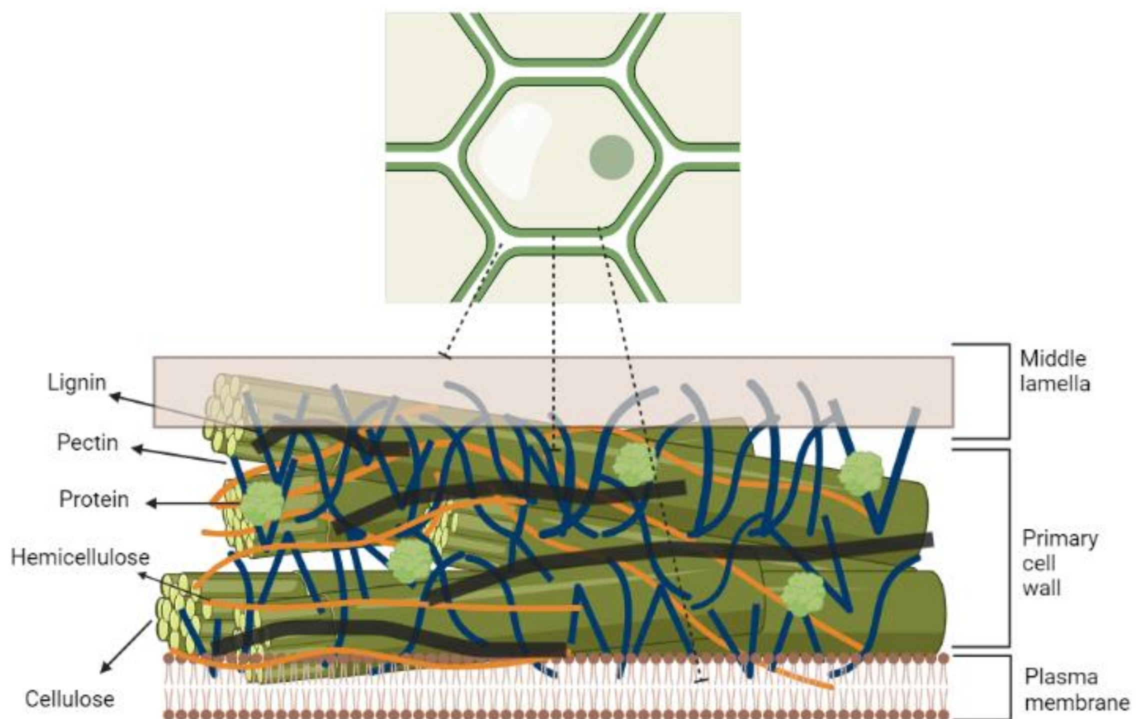

Figure 2.2. Three-dimensional view of polymer arrangement in the plant cell wall. Created with BioRender.com based on information in Cui et al. (2021) with permission from Elsevier.

Generally, the chemical structure of pectin is diverse and changes with the extracting source. However, three well-known structures are established as homogalacturonan (HG), rhamnogalacturonan I (RG-I), and rhamnogalacturonan II (RG-II) in pectin structure. While HG is considered as smooth region with linear chain of D-galacturonic acid units partially esterified with methanol and acetyl groups, RG-I and RG-II are considered as hairy regions substituted with different side chains containing neutral sugars such as galactose, rhamnose, arabinose. The side chains of RG-I may contain around 40 different structures (Reichembach and Petkowicz 2021). Mainly galactosyl and/or arabinosyl residues along with varying amounts of the rhamnosyl units (25-80%) are substituted in this region (Voragen et al. 2009). Besides, RG-II is known to be the most complex and conserved structure probably composed of a short galacturonan chain substituted with five structurally different side chains linked to 13 different types of sugars (Reichembach and Petkowicz 2021; Voragen et al. 2009). The proportion of HG varies from 60-65% while the 20-35% of the total pectin is accounted for RG-I and the rest for RG-II (Ngouémazong et al. 2015). Also, as pectic polysaccharides are accepted to link each other with glycosidic bonds in the cell wall, they may be covalently linked to other components such as xylan, cellulose, phenolics, proteins or ionic crosslinked with calcium cations (Reichembach and Petkowicz 2021). Thus, the unique structure of each pectin is required to be well investigated and its functional properties should be revealed in order to use pectin in industry.

2.2.2. Pectin Sources

Pectins can be extracted from various plants from different sources but their yields, structural and functional properties differ. Some of the plant sources used for pectin extraction together with their basic molecular properties, reported yields and relative functions found in literature over the last years are summarized in Table 2.2. On commercial scale, pectin is produced mainly from by products (peels or pomace, etc.) of fruits such as citrus (85%), apple (14%), or sugar beet (0.5%) with hot dilute acid at 70-

90 °C and pH 1.0 to 3.5 and marketed with the EU code E440 as a food additive with ‘generally recognized as safe’ (GRAS) status referred as used in food at levels not to exceed good manufacturing practices (FDA 2023; Ciriminna et al. 2016). According to the Fruit processing report, the value of the global pectin market is expected as $1.8 billion with a 7.6% growth by 2026 (Fruit Processing 2020). The fact that the pectin demand in the global market exceeds 30,000 tons per year shows that alternative pectin sources will create potential (Ciriminna et al. 2016).

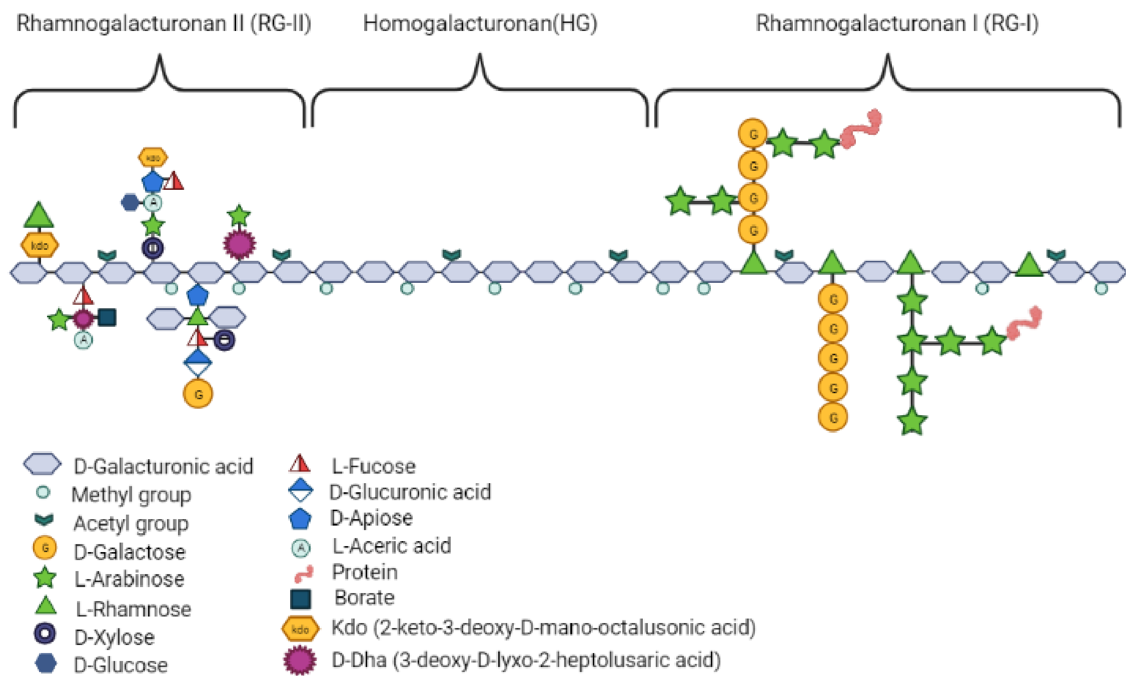

Figure 2.3. Schematic representation of the structure of pectin. Created with BioRender.com based on information in Reichembach and Petkowicz (2021) with permission from Elsevier.

Table 2.2. Structural characteristics and functional properties of pectins extracted from various sources with different methods.

Source	Yield (%)	GA (%)	DE (%)	Type of extraction	Extraction Condition	Properties	Reference
Citrus peel	16.7-28.4	24.08	n.d.	HAE	Acid: Nitric, oxalic. Temp (ºC):70,72,85. pH:1.6, 2.1, 3.5, 4.6. Time (h): 3, 7, 1.5, 2.5.	Rheological	(Kaya et al. 2014)
Apple (fresh)	16	65	57	HAE	Acid: Citric. Temp:100 ºC. Time (min):30.	Rheological, Gelation	(Rascón-Chu et al. 2009)
Apple pomace	5.5–19.6	69.30-75.90	59.08-82.66	HAE	Acid: Acetic, sulfuric, hydrochloric, nitric. Acid conc.:5-40% (w/w) for AA, pH 2.4 for mineral acids. SLR: 1:25. Temp:80-110 ºC. Time (min): 50, 70, 90, 110, 130.	Rheological	(Luo, Xu, and Fan 2019)
Sugar beet pulp	5.5-17.2	52.7-78.8	45-71	HAE	Acid: Citric, malic, lactic, hydrochloric. Temp:80 ºC. pH: 1.5, 2. Time (h):1, 2.	Emulsification	(Ma et al. 2013)
Sunflower seed head	0.2-15.6	79.7-92.6	15-49	HAE	Acid: Sodium citrate, nitric. Temp: 50-80 ºC. SLR: 0.3-1% for sodium citrate and 0.01-0.2% for nitric acid. Time (min):80-240.	Emulsification, Rheological, Gelation	(Muñoz-Almagro et al. 2018)
Apple pomace	15.3-19	61-70	57.3-58.7	EAE	Enzyme: Cellulase. Dose: 25, 50, 75 µL/g of pomace. Temp:50 ºC Time (h):18.	Antioxidant, Antitumor	(Wikiera et al. 2015)
Chicory roots	3.8-34.6	49.5-64.8	31-66	HAE, EAE	HAE: Hydrochloric, pH 1.3, 85 ºC, 3h. EAE: Cellulase, protease, pectinase. Dose: 1:10, 1:100 (v/v). pH: 5.5, 40 ºC. Time (h): 4-48.	*	(Panouillé, Thibault, and Bonnin 2006)

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Table 2.2 (cont.)

Source	Yield (%)	GA (%)	DE (%)	Type of extraction	Extraction Condition	Properties	Reference
Opuntia ficus indica cladodes	10.36-17.92	66.66	35.04	EAE	Enzyme: Cellulase, xylanase. SLR:10, 20, 30 ml/g, the cellulase/xylanase ratio:2, 3, 4 U/U. Enzymes/material ratio:2, 3, 4 U/g. pH: 5.0, 5.5. Temp:50, 55, 60 ºC. Time (h):3, 6.	Functional, Emulsification, Antioxidant	(Bayar, Friji, and Kammoun 2018)
Artichoke (Cynara scolymus L.) By-products	9.81-20.83	3.05-5.81	n.d.	EAE	Enzyme: Cellulase. Powder conc.: 2-7% (w/w). Dose: 2.2–13.3 U/g. pH: 5.0. Temp: 50 ºC. Time (h):6-24.	*	(Sabater et al. 2018)
Lime peel	13.6-26.3	81.3-90.4	67.3-82.2	HAE, EAE	HAE: Nitric, pH: 1.7 or 2, 70 ºC, Time (h): 8 or 4. EAE: Cellulase, hemicellulase (including xylanse, arabinoxylanase). Dose: 1.875 µL/mL. pH: 3, 3.5, 4, 4.8. Temp:50 ºC. Time (h):4.	Stabilization, Gelation, Sensitivity	(Dominiak et al. 2014)
Tomato paste	15.01-36	24.95-57.2	76.92-88.98	HAE, UAE	HAE: Ammonium oxalate (16 g/L)/oxalic acid (4 g/L) under reflux, 60-80 ºC, 24 h and 12 h. UAE: Ultrasonic bath with 37 kHz. Temp: 60 and 80 °C. Time (min): 15, 30, 45, 60, 90.	*	(Grassino et al. 2016)
Grapefruit peel	23.50-27.46	50.03-55.20	65.52-67.59	HAE, UAE	HAE: Hydrochloric, pH: 1.5, 80 ºC, 90 min. UAE: Ultrasound probe with 20 kHz at 800 W. Pulse: 50% (2 s on: 2 s off). Power intensity: 10.18, 12.22, 14.26 W/cm^2. Temp: 60-80 ºC. Time (min): 20-40.	Rheological, Antioxidant	(Wang et al. 2015)

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Table 2.2 (cont.)

Source	Yield (%)	GA (%)	DE (%)	Type of extraction	Extraction Condition	Properties	Reference
Mango peel	1.55-17.5	29.35-53.35	85.43-88.38	HAE, UAE	HAE: Citric, SLR:1:40, pH: 2.5, 20 and 80 ºC, 2h. UAE: Ultrasound probe with 20 kHz at 500 W. Pulse duration: 5 s on 5 s off. pH: 2.5, Temp: 20 and 80 ºC. Time (min):15.	Emulsification, Rheological	(Wang et al. 2016)
Sisal waste	6-31	52-63	33-50	HAE EAE, UAE, UEAE, EUAE	HAE: Hydrochloric, pH 1.5, SLR:1:20, 100 ºC, 90 min. EAE: Cellulase, SLR: 1:15, 88 U/g, 50 ºC, pH 4 for 20 h. UAE: Ultrasound probe with 20 kHz at 450 W. Ammonium oxalate (0.6%). SLR: 1:15. Time (min):60.	Rheological	(Yang et al. 2018)
Passion fruit peel	7.53-12.67	66.27-76.29	60.36-79.59	HAE, UAE	HAE: Nitric, SLR:1:30, pH: 2.0, 85 ºC, 10 min. UAE: Ultrasound probe with 20 kHz at 664 W. Power intensity: 132.8-664 W/ cm^2. pH:2.0, nitric acid, SLR:1:30. Temp:45-85 ºC. Time (min):10.	*	(Cladera-Olivera et al. 2016)
Gold kiwifruit	1.01-4.39	28.96-58.57	82-90	HAE, HWE, EAE	HAE: Citric acid, pH2.20, 1:6 SLR, 50 ºC, 60 min. HWE: Reverse osmosis, pH 3.50-3.60, 1:4 SLR, 25 ºC, 30 min. EAE: Cellulase, 1.05 mL/kg, 25 ºC, 30 min.	Rheological	(Yuliarti, Matia-Merino, et al. 2015)

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Table 2.2 (cont.)

Source	Yield (%)	GA (%)	DE (%)	Type of extraction	Extraction Condition	Properties	Reference
Pistachio green hull	3.7-11.8	59-75	41-60	HWE, UAE	HWE: pH 6.5, Temp:70, 80, 90 ºC. Time (min): 30, 60, 90. SLR: 20, 30, 40. UAE: Ultrasound probe. Power: 90, 120 ,150 W. pH:1.5, 2.25, 3. SLR:1:25. Time (min):10, 20, 30.	Functional, Emulsification, Antioxidant	(Kazemi et al. 2020)
Grapefruit	17-31.88	68.21-74.86	75.6-82.61	MAE, UAE, HAE, UMAE	MAE: Oven with 900 W and 2450 MHz, Hydrochloric, pH 1.50, Power (kW): 0.45, 0.63, 0.9. 1:50 SLR, Time (min): 2, 4, 6, 8, 10, 12, 14. UAE: Ultrasound probe with 24 kHz at 200 W, Pulse: 30 s on 30 s off, Hydrochloric, pH 1.50, 1:50 SLR, Temp: 50,60,70 ºC, Time (min): 4, 12, 15, 20, 25, 30. HAE: Hydrochloric, pH 1.50, 90 ºC, 90 min, UMAE: UAE-water, pulse: 30 s on 30 s off, MAE-Hydrochloric, 0.45 kW, 8min	Rheological	(Bagherian et al. 2011)
Pumpkin	1-11.3	51-58.9	52.1-63	HAE, MAE	HAE: Hydrochloric, 65 ºC, 2 h. MAE: Oven with 1200 W and 2450 MHz. Temp.:60, 80, 120 ºC. Time (min): 3, 10, 20.	Rheological	(Yoo et al. 2012)

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Table 2.2 (cont.)

Source	Yield (%)	GA (%)	DE (%)	Type of extraction	Extraction Condition	Properties	Reference
Hawthorn wine pomace	61.01-72.89	65.93 - 72.24	8.84-37.95	HAE, MAE, EAE	HAE: Citric acid or Hydrochloric, SLR: 1:6, pH: 2.5 or 1.5, Temp: 85 ºC or 95 ºC, Time (h): 1.5 or 2. MAE: Oven with 440 W. Sodium hexametaphosphate (1.35%, v/v). SLR: 1:9. Time: 80 s. EAE: Cellulase. SLR: 1:8, pH 5.0. 80 U/g. 60 ºC, 4 h.	Rheological, Antioxidant, Gelation	(Sun, Chen, and Zhu 2020)
Eggplant peel and calyx	18.36-29.17	60.2-67.4	60.74-68.18	MAE	MAE: Oven with 700 W. pH:1.5. SLR: 1:20. Time: 2 min.	Functional, Emulsification, Antioxidant	(Kazemi, Khodaiyan, and Hosseini 2019)
Watermelon rind	13-16.01	27.18-88.16	30-46	HAE, MAE	HAE: Hydrochloric, 90 ºC, pH 1.5. SLR: 1:20, 150 min. MAE: Oven with 2450 MHz. pH:1.5, SLR:1:20, Power: 100, 300, 500W. 90 ºC. Time (min): 5, 14, 26.	Functional, Gelation	(Majid et al. 2023)
Cacao pod husk	7.4-11	48.9-59.5	36.8-42.2	HAE, SWE	HAE: Citric 4% (w/v), 95 ºC, pH 3. SLR: 1:25, 95 min. SWE: 121 ºC, 103.4 bar, 30 min.	*	(Muñoz-Almagro et al. 2019)
Bay tree pruning waste	8.74-21.76	0.1-47.4	0.07-50.64	SWE	SWE: tap water, pH 7.3, Temp.:100, 120, 140, 160 ºC. Time (min): 5, 10, 15, 20. SLR:1:8.	Film-forming	(Rincón et al. 2021)
Jackfruit peel	13-15	52-57	61-74	HAE, SWE	HAE: Citric, 90 ºC, pH 2. SLR: 1:30, 2 h. SWE: Temp: 130, 135, 140 ºC, Time (min): 5, 7.5, 10. SLR: 10, 15, 20.	Rheological, Gelation	(W. Li et al. 2019)
Olive pomace	1	11-42	5-19	MemE	Ultrafiltration with 3 kDa	Functional, Emulsification, Antioxidant	(Rubio-Senent et al. 2015)

n.d.: not determined, Temp: Temperature, SLR: solid liquid ratio; HAE: Hot acid extraction; EAE: Enzyme-assisted extraction; UAE: Ultrasound-assisted extraction; MAE: Microwave-assisted extraction; HWE: Hot water extraction; SWE: Subcritical water extraction; UEAE: Ultrasound- and enzyme-assisted extraction; UMAE – Ultrasound- and microwave-assisted extraction; MemE: Membrane extraction.

2.2.3. Pectin Extraction

Pectin extraction is a combined process of hydrolysis of protopectin from cell wall by using solvents with or without several equipment and isolation of the resultant pectin from the solution (Adetunji et al. 2017). The success of the extraction is based on several factors including mainly temperature, pH of the extracting medium. Besides, equipment related conditions such as optimal operating conditions, source related factors such as pretreatment, particle size, moisture, and extrinsic factors based on extracting medium such as solid liquid ratio, polarity, volatility, toxicity are the other factors affect both the yield and physicochemical properties of pectin. Extreme operating conditions such as high operating temperature or pH must be avoided during extraction due to lability of pectin to these factors that ended up depolymerization and deesterification with poor functionality product (Lopes da Silva and Rao 2006). Time is also another important factor that needs optimization during extraction. The transfer of pectin from the cell wall to the extracting medium increases the viscosity and with time this reduces the transfer rate. Also, degradation may happen with continuous long treatment in extraction. Over dilution or less use of solvent must be avoided due to decrease hydrolysis of pectin (Kertesz 1951). Generally, before extraction, the raw materials are subjected to washing process to remove dirt or unwanted materials with water or alcohol and dried (Roman-Benn et al. 2023). Then, to enhance the extraction efficiency, they are cut to the desired size to increase the contact area, and the solid-liquid ratio is adjusted to achieve sufficient agitation that can lead to a high yield of pectin with superior quality (Adetunji et al. 2017). In Table 2.2, changing yields and functionalities can be obtained depending on the raw material and extraction conditions. Roughly, extraction methods are named according to the solvent or equipment used. The conventional method uses acid with low pH and high temperatures to be named hot acid extraction (HAE). The multiple methods such as enzyme-assisted extraction (EAE), ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), subcritical water extraction (SWE) have significantly upgraded in recent years and utilized both single and combined in numerous studies thanks to the development of technology and the spread of environmental awareness (Table 2.2). Besides, alternative approaches such as the moderate electric field (MEFE)

and the ohmic heating-assisted (OHAE) extractions are found in the literature as innovative and advanced methods (Reichembach and Petkowicz 2021).

As previously mentioned, commercially, pectin is extracted by chemical method based on acid hydrolysis at high temperature over a long time. Basically, this process consists of two main stages. The first stage is the extraction process with hot dilute acid from the plant raw material. Then, in the second step, the extracted pectin is isolated and purified (Methacanon, Krongsin, and Gamonpilas 2014). In order to isolate (or precipitate) pectin, ethyl alcohol is used to remove sugars, some acids and many other compounds and to improve color. The precipitated pectin is collected and washed several more times with ethanol to increase purity. Finally, the pectin collected by centrifugation or filter is dried and milled to make powder. Drying is carried out at low temperature, under vacuum or by using freeze drying methods (Kertesz 1951). High efficiency acid extraction uses mineral acids such as sulfuric, hydrochloric, phosphoric and nitric acid. The use of mineral acids is cheaper and more effective in terms of production yield. However, the fact that mineral acids have a corrosive structure that negatively affects human and environmental health, the use of organic acids in pectin production is more sustainable (Ma et al. 2013). For this purpose, organic acids such as citric, tartaric, lactic, acetic, oxalic acid and ammonium oxalate and polyphosphate are used (Srivastava and Rishabha 2011). In particular, the use of citric acid in pectin extraction has been reported to be more effective than the use of hydrochloric, nitric, phosphoric and sulfuric acids with organic acids such as tartaric, acetic and lactic acid (Abid et al. 2017). Citric acid is preferred for pectin production with its economical and less damage to the environment (Raji et al. 2017). Other factors affecting acid extraction are extraction temperature, time and pH value (Methacanon, Krongsin, and Gamonpilas 2014). In hot acid extraction (commercial), the processing conditions are pH 1.4-3, temperature 50-100 °C and time can vary from 20-360 min. These parameters vary according to the raw material, the desired pectin type, the targeted yield or the capacity of the enterprises (Minjares-Fuentes et al. 2014). The final pH of the medium in acidic extraction is highly effective on yield. In literature, it was seen that when the pH of the environment was 2.0 and this increased the efficiency of pectin extraction (Muhammad et al. 2014; Yuliarti, Goh, Matia-Merino, et al. 2015; Yuliarti, Matia-Merino, et al. 2015). In addition, the temperature must be applied to a degree and a suitable time so as not to cause further hydrolysis and modification of the pectin.

Enzymes such as cellulase and hemicellulase are also used to increase yield in pectin extraction (Panouillé, Thibault, and Bonnin 2006; Vasco-Correa and Zapata Zapata 2017). The purpose of these enzymes is to extract pectin which is bound to the cellulosic matrix or trapped within this matrix. However, it is important to see whether there is an undesirable change in physicochemical properties of extracted pectin, when enzymatic extraction method is used (Panouillé, Thibault, and Bonnin 2006). One of the effective enzymes used in extraction of pectin is protopectinase which is a microbial enzyme that allows the dissolution of pectin from the protopectin. Vasco-Correa and Zapata Zapata (2017) used protopectinase to extract pectin from passion fruit and determined that the level of galacturonic acid increased from 15.9 g/100 g to 17-25.8 g/100 g. In another study, cellulases were used in three different concentrations (0.1 mL/kg, 1.05 mL/kg and 2.0 mL/kg) for obtaining pectin from kiwi fruit. The highest yield (8.08%) was obtained with 1.05 mL/kg enzyme used (Yuliarti et al. 2011). In another study in which pectinases and cellulases were used together to facilitate the dissolution of pectin from bergamot peels, it was reported that cellulase alone dissolved 62% of the peel (Mandalari et al. 2006).

In the ultrasound-assisted extraction, the sound waves that the human ear cannot hear cause air bubbles and negative pressure in the liquid environment, causing the material to swell and become more porous. This causes better mixing of the molecules with the solvent and increases the efficiency by increasing the mass transfer (Naqash et al. 2017). In this way, the amount of solvent used in the ultrasound-assisted extraction is reduced as well as the wastes from organic solvents and the extraction time is shortened. In a study on the extraction of pectin from grapefruit fruit using ultrasound method, the extraction time decreased by 38% while the extraction efficiency increased by 16% (Wang et al. 2015). In addition, it can alter the physicochemical properties of the pectin, such as the bioactive profile and gelation, because the ultrasound partially disintegrates polymers. As a matter of fact, as the ultrasound intensity and application time increased, the gelation rate of pectin decreased but more transparent appearance gels were formed (Zhang et al. 2013). Therefore, it should be taken into consideration how much the targeted properties of pectin change when applying both enzymatic and ultrasound assisted extraction.

A microwave is also used to increase the yield in pectin extraction. Microwave-assisted extraction uses heat to heat the solvents in contact with plant tissue. As a result,

with the microwave application, the solvent penetrates better into the plant material and increases the solubility of pectin. The process time is shortened as the warm-up takes place rapidly (Adetunji et al. 2017). It was determined that the yield of pectin from orange peel was increased by 14% with microwave application, whereas the molecular weight and viscosity values of pectin decreased slightly (Guo et al. 2012). Considering all these, it is obvious that the yield enhancing techniques should be used in such a way that pectin does not lose its target functional characteristic at the end of the extraction.

The subcritical water extraction is indicated as a eco-friendly method for producing pectin from wastes (Li et al. 2019). The extraction medium is water at liquid state operating under the high pressures as 1-22 MPa and temperatures as 100-374 ºC (Mustafa and Turner 2011). This condition leads to altering the dielectric constant with decreasing values (from 79 at 25 ºC to 33 at 200 ºC) and lose the hydrogen bond of cell wall matrix to increase the solubilization of pectin within a short time (Muñoz-Almagro et al. 2019; Adetunji et al. 2017). Recently, Cui et al. (2022) used subcritical water to extract pectin from persimmon peel with 7.71% yield at 140 ºC and 3 min of operation showing good antioxidant and prebiotic activities. Similar to the processing conditions of this study, Li et al. (2019) was compared subcritical water with hot acid extraction to obtain pectin from jackfruit peel waste. Where the former gave the maximum yield as 14.96% (138 ºC, 9.15 min, L/S ratio 17.03 mL/g), the latter gave 16.83% (90 ºC, 2 h, L/S ratio 30:1). Besides, SWE exhibited higher pectin yield, higher galacturonic acid content and higher degree of methyl esterification than HAE by producing high purity pectin from cacao pod husk (Muñoz-Almagro et al. 2019). Thus, these results provide the utilization of SWE for the efficient pectin production with enhanced functionality by saving both time and environment.

The current use of the commercial hot acid extraction method for industrial pectin production is well established. It is also used as a reference for comparison purposes with other extraction methods. Due to high reproducibility, it allows scale-up. Advantageously, it requires lower initial investment and technology input compared to other methods. However, its sustainability is low due to the creation of chemical pollution and its energy consumption. On the contrary, enzyme-assisted extraction is a very low energy consuming method. The initial investment cost is relatively low. However, considering the cost related to the enzymes used and long extraction time reduce the overall cost-effectiveness. The other mentioned above technologies as microwave,

ultrasound-assisted and subcritical water extraction need special technologies and employers to operate along with high initial investment cost which offer limited scale-up. However, the short extraction time and high yield even up limitations. Cost, energy consumption and productivity are important targets for the industry in choosing the pectin extraction method. Growing pectin demand leads the improvement of this process towards fast, feasible, repeatable and greener approaches. It is obvious that the yield enhancing techniques should be used in such a way that pectin does not lose its target functional characteristic at the end of the extraction.

2.2.4. Functional Properties Used in Food Applications

Commercial pectin is mainly used in industry as a gelling agent, water holding and thickening agent and as an edible film and coating agent in jams, yogurt products, fruit milk beverages, canned foods, confectionery and confectionery products, sauces and ice cream (Espitia et al. 2014; Noreen et al. 2017). The physicochemical properties and functionality of pectin rely on primarily with its structural characteristics such as molecular weight, galacturonic acid content and degree of methyl-esterification and spatial conformation such as RG-I/HG ratio (Cui et al. 2021). Based on the literature summarized above in Table 2.2, one thing is certain that the extraction method significantly changes the structural properties and, accordingly, the functionality of pectin.

2.2.4.1. Gelation

The most well-known property of pectin is its gel formation. Generally, based on the carboxyl groups of the galacturonic acid units in the pectin straight chain esterified greater or less than 50% with methyl alcohol, pectin is classified as high-methoxyl (HMP) or low-methoxyl (LMP), respectively (Abid et al. 2017). HMP gel is produced by heating and cooling the pectin solution in the presence of acid ($pH < 3.5$) and cosolute (sucrose, >55%). In the presence of high concentration of cosolute, the water activity reduces to allow chain-chain interactions rather than chain-solvent interactions. Also, protonation of the carboxyl groups with the low pH helps to maintain junction zones by forming intermolecular hydrogen bonds between carboxyl and hydroxyl groups as well as hydrophobic interactions between methyl-ester groups of adjacent GA units (Muñoz-Almagro, Montilla, and Villamiel 2021). This hydrophobic effect between methyl esters repels the water from the pectin chains resulting a coalescence which stabilizes the HMP gels (Chan et al. 2017). On the contrary, LMP gels in the presence of low sugar and divalent cation (calcium, Ca^{++}) in wide range of pH (2-6). The gel network of LM pectin is formed by the ionic crosslinks via calcium bridges between two carboxyl groups of neighboring chains. This mechanism is referred to as the "egg box" model due to more ordered side by side associations of the pectin chains with the Ca^{++} (Chan et al. 2017). There must be at least 6 (up to 20) non-methoxylated galacturonic acid units oriented to form junctions of the egg box (Fraeye, Colle, Vandevenne, Duvetter, Buggenhout, et al. 2010). Tuning the DE by acid or alkali treatment (demethoxylation, depolymerization) or with this manner increases the gel strength of LMP. Also, enzymes such as pectin methyl esterase can be used as alternative methods to prevent depolymerization. If demethoxylation runs in the presence of ammonia, amide groups replace the ester groups to form LM-amidated pectins. These pectins need minor amount of calcium to induce gelling due to contain less amounts of charged GA units (Fraeye, Duvetter, et al. 2010). The free or esterified distribution of GA units in the LMP structure affects the binding power of Ca^{++} and the functionality of the pectin gels (Fraeye, Duvetter, et al. 2010; Thakur, Singh, and Handa 1997). On gelation of HMP, the blockwise distribution of methyl-esterified GA units set as a faster rate with the same DE value of randomly

distributed methyl-esters (Chan et al. 2017). There is a correlation between the degree of esterification and the gel setting rate that over 70% esterification, HMP can be called as "rapid-set" pectin whereas near 60% esterification, HMP can be classified as "slow-set" pectin and "medium-set" pectin in between (May 1990; El-Nawawi and Heikel 1997; Thakur, Singh, and Handa 1997). The main difference between rapid-set pectin and slow-set pectin is that the latter needs a lower pH to form gel with similar strength (El-Nawawi and Heikel 1997). HMP is suitable for regular jam, marmalade and jelly production whereas LMP can be used in low-calorie foods, diet, jam, marmalade and jelly (Einhorn-Stoll 2016). In such food formulations, polyols such as glycerol, xylitol, sorbitol and erythritol or other simple sugars as glucose, fructose can be used as cosolute to replace sucrose according to the target (Chan et al. 2017; Reichembach and Petkowicz 2021). Gelation was occurred in the presence of 75 mg of Ca^{++} and 1% concentration of LM sunflower seed head pectin by showing no difference between the sugar concentrations of 20-40% (Muñoz-Almagro et al. 2018). The existence of protein together with high GA content resulted in high gel strength of microwave-assisted extracted LM pectin from watermelon rind (Majid et al. 2023). The decrease in GA and DE contents as well as the MW value caused a week gelation of HM jackfruit peel pectin extracted with SWE (W. Li et al. 2019). However, LMP obtained from hawthorn wine pomace with EAE performed best gelation compared to pectins extracted with HAE and MAE within the study due to lower DE value to form calcium bridge (Sun, Chen, and Zhu 2020). Also, EAE from the lime peel resulted with high yield HMP showing functionality as gelation with increased calcium sensitivity and ability to stabilize acidified milk drinks (Dominiak et al. 2014). Beyond the GA and DE content, the acetyl content over the 4% of pectin inversely affects the gelation (Voragen, Schols, and Pilnik 1986). The poor gelation of sugar beet pectin has been reported due to acetyl groups present (Dea and Madden 1986; Hao Chen et al. 2018; Pacheco et al. 2019). However, mixture of 60% sucrose, 1.32% cacao pod husk pectin (GA equivalent, w/w) at pH 2.5-3.3 was formed gel despite the 17.1% degree of acetylation (Vriesmann and Petkowicz 2013) due to differences in distribution of the groups within the pectin structure.

2.2.4.2. Thickening

Pectin is used extensively as a thickening and water-retaining agent in many foods. Pectin dissolves in water without heating to form a viscous liquid. When the liquid-mass ratio of the pectin-water solution was examined, it was observed to be almost Newtonian, semi-liquid, or gel-like (Einhorn-Stoll 2018). The viscosity of pectin solutions depends not only on the polymer concentration but also on the molecular weight of the polymer and the pH of the medium and the ionic interactions occurring in the medium. However, as the molecular weight generally increases, the viscosity of pectin solutions increases (Abid et al. 2017). It is observed that pectin extracted with hot acetic acid from apple pomace was high in MW (119.57 kDa) and DE (82.66%) that resulting the stronger inter- and intra-molecular with 1.5-fold higher viscosity than mineral acid extracted apple pectins (MW= 93.2-105.4 kDa, DE= 77.5-80.5%) (Luo, Xu, and Fan 2019). Increase in temperature from 20 ºC to 80 ºC led to higher MW (664.5-2320 kDa) during ultrasonic extraction of HM mango peel pectin resulting 5-fold increase in apparent viscosity (6-30 mPa.s) (Wang et al. 2016). The concentration dependent increase in viscosity is observed widely in literature such as cacao pod husk pectin (Vriesmann and Petkowicz 2013), citrus peel pectin (Sousa et al. 2015), dragon fruit peel pectin (Muhammad et al. 2014) and pomelo peel pectin (Methacanon, Krongsin, and Gamonpilas 2014). The intermolecular distance becomes less when more pectin molecules present in the solution leading to formation of more hydrogen bonding resulting increase in viscosity up to a certain point changing with the pectin used (Chan et al. 2017; Sousa et al. 2015; Muhammad et al. 2014). That point is stated as 5% pectin concentration in the study by Einhorn-Stoll (2018). After that concentration, the Newtonian behavior may turn to shear thinning (non-Newtonian or pseudoplastic) behavior depending on the molecular weight of pectin (Chan et al. 2017). However, the sisal pectin solutions showed shear thinning behavior below the stated point (0.5%-2%) and tended to be more obvious with increasing concentration suggesting weak network in aqueous solution (Yang et al. 2018). The pectin concentration used in fruit beverages, soft drinks, sauces, and sorbets is limited between 0.01 and 0.5% due to viscosity effect (Flutto 2003).

2.2.4.3. Emulsification

The intrinsic properties of pectin such as presence of protein or ferulic acid, acetylation, degree and conformation of methyl esterification, molecular weight and sugar composition directly affect the emulsifying ability. Basically, the emulsification capacity of pectin relies on three main mechanisms as steric hindrance, electrostatic repulsion, and viscosity of solution (Ngouémazong et al. 2015; Dickinson 2003). Covalently bound protein moiety of the pectin chain plays a dominant role in pectin emulsification that creates the thick hydrated layer around the oil droplets causing steric stabilization (Dickinson 2003). The contents of ferulic acid, acetyl, methyl ester groups or sugar side chains on the pectin structural conformation, also support the hydrophobic interaction with the adsorption on oil droplet and reduce interfacial tension (Alba and Kontogiorgos 2017). Meanwhile, the negatively charged hydrophilic part of the pectin chains due to its unmethylated GA groups extends into the aqueous phase like a tail (Ngouémazong et al. 2015). Thereby, both the steric and electrostatic repulsion mechanism work under these influences to stabilize the newly formed oil droplets against droplet coalescence, flocculation during storage. In this context, sugar beet pectin is become very famous with its good performance on emulsification owing to its high protein, acetyl and ferulic acid contents (Chen et al. 2018; Liu, Guo, and Meng 2020; Chen, Fu, and Luo 2016; Williams et al. 2005; Leroux et al. 2003). As it is known, pectin can increase viscosity of the solution or form gel network based on the other constituents in the solution that result positive or negative effect on emulsification or emulsion stabilization. Increase in viscosity reduces the mobility of dispersed oil droplets within the aqueous phase and promotes stability during storage (Dickinson 2003; Leroux et al. 2003). In fact, the effect of MW is contradictory. The low molecular weight of pectin may inhibit the emulsifier performance compared to high molecular weight pectins due to form a thin layer around the oil droplet resulting instability problems (Alba and Kontogiorgos 2017). However, they may show fast adsorption to the droplet surface promoting the stability quicker than high MWs (Schmidt et al. 2015). The high MWs are able to make more intermolecular interactions that form aggregates to stabilize the oil/water interface (Leroux et al. 2003; Ngouémazong et al. 2015). In their study, Funami et al. (2011) showed that decrease in

molecular weight of sugar beet pectin might be related to losing hydrophobic moieties such as protein, methyl ester or ferulic acid groups resulting less stability on emulsification. In contrast, ultrasonic degradation occurred in the structure of pistachio green hull pectin more than HWE (1.3-fold high GA content) resulting better performance on emulsification (Kazemi et al. 2020). Likewise, emulsification can be triggered or inhibited by extrinsic factors such as polymer concentration, pH of the solution. The decrease in the pH value of the solution below the pKa of GA (3.5) causes protonation of ionizable carboxylic groups that provide a steric stabilization by forming a compact thick structure at the oil-water interface of the pectin chains (Einhorn-Stoll 2018; Alba and Kontogiorgos 2017). This situation observed in okra pectins that at pH 2 viscosity of emulsion increased as a result of groups come into close contact resulting lower droplet diameters than pH 6 emulsions (Alba, Sagis, and Kontogiorgos 2016). Similarly, Schmidt, Schütz, and Schuchmann (2017) measured an emulsion in a layer twice as thick as that of freely suspended pectin molecules at pH 2. In contrast, above high pH (pKa < pH), since the increase in the number of negatively charged groups due to deprotonation can cause intramolecular electrostatic repulsion that subsequently cause and open conformation of the pectin structure (Alba and Kontogiorgos 2017). The more negatively charged groups observed at pH 7 of the citrus pectin emulsions than pH 3 emulsions (Verkempinck et al. 2018). Apart from being the pectin is used as a single emulsifier, different biopolymer complexes also employed in literature for the emulsification such as with protein (Dalev and Simeonova 1995; Xu et al. 2012), enzyme (Littoz and McClements 2008), chitosan (Niu et al. 2019) or used in Pickering emulsion (Jiang et al. 2020).

2.2.4.4. Edible Film and Coating Formation

Edible coatings are basically known as the thin layer of eatable materials that cover directly on the food surface and give some barrier properties against such as moisture, oxygen. On the other hand, edible film is referring as the self-standing structure

which has the thickness less than 254 µm (Yemenicioğlu 2022). They both are the promising alternatives to plastic packaging. Pectin has been proved to be a good biodegradable, environmentally friendly food packaging agent that can be obtained from various sources (Kumar et al. 2020; Mellinas et al. 2020; Huang et al. 2021; Valdés et al. 2015; Espitia et al. 2014). As it well known that pectin-based coatings are inferior against water vapor and superior at gas permeation due to hydrogen bonded network causing hydrophilicity (Baldwin, Hagenmaier, and Bai 2012). Several active agents as antimicrobials, antioxidants, nanoparticles or nutrients can integrate the pectin-based coating or film forming formulations to increase the shelf life, nutritive or sensory characteristics and textural properties (Rojas-Graü, Soliva-Fortuny, and Martín-Belloso 2009). Recently, composite films made from red pomelo peel pectin combined with casein and egg albumin. It was found that pectin enhanced the film forming performance of proteins by increasing the compactness of film structure and thermal stability as well as decreasing the elongation of films (Sood and Saini 2022). Incorporation of candelilla wax to the emulsion-based film increased the water vapor permeability by 7.4-fold of the hawthorn fruit pectin film (Lozano-Grande et al. 2016). Another emulsion-based pectin film successfully achieved by adding clove bud essential oil to the citrus pectin film (Nisar et al. 2018). Developing films not only showed antimicrobial activity against three foodborne pathogens as *Escherichia coli*, *Staphylococcus aureus* and *Listeria monocytogenes* but also improved the water vapor resistance and thermal stability. Plant extract contains a lot of phenolic compounds that would be advantages to use in edible films. Along with the water vapor permeability, antioxidant capacity of the mango peel pectin film increased by addition of polyphenol-rich extracts from Tommy Atkins mango peels (Ribeiro et al. 2021). The citrus pectin-based films developed blended with mulberry leaf extract containing mainly deoxynojirimycin and chlorogenic acid resulting high antioxidant, antimicrobial and better mechanical property films (Shivangi et al. 2021). This film was used for coating capsicum fruit to double shelf life compared to control fruit. Another approach that used plant phenolics to use fruit juice as a film forming aqueous phase as in the study by (Azeredo et al. 2016). Pomegranate juice was used both for the aqueous phase and as plasticizer in apple pectin films with citric acid. The developed films showed good anthocyanin-pectin interaction that had improved elongation. The bright red color could be useful in coatings of fruit strips or wraps for sushi-like products. Apart from developing colorful films, preventing the food from the UV-light may also be needed in food packaging to decrease the chance of lipid oxidation,

discoloration (Farris, Introzzi, and Piergiovanni 2009). Recently, Hari et al. (2021) successfully developed UV-barrier film with pectin and zinc oxide nanoparticles which also showed excellent antibacterial effects against both E. coli and S. aureus by inhibiting 2-log. Another approach that used pectin films as carriers to probiotics. Pectin and whey protein isolate mixed to encapsulate *Lactobacillus rhamnosus* GG in a thin layer of film. Sodium alginate, low esterified amidated pectin, kappa-carrageenan/locust bean gum and gelatine in the presence or absence of whey protein concentrate were used to prepare film matrix to carry *L. rhamnosus* GG for the delivery (Soukoulis et al. 2017). *L. rhamnosus* GG showed highest cell count after drying with pectin/whey protein concentrate film forming systems. Another study using alginate with pectin combination to make hydrogel films to encapsulate a cholesterol-lowering antihyperlipidemic agent, simvastatin, for wound healing application was conducted by Rezvanain et al. (2017). It was proved that by crosslinking with Ca^{++} the mechanical strength of alginate/pectin film increased resulting in good wound fluid uptake and slow drug release behavior.

2.2.5. Non-food Applications

Besides, pectin is used in various applications in tissue engineering, wound healing, gene transfer, drug transport, cancer treatments (Gilani et al. 2008; Rezvanain et al. 2017; Zhang, Xu, and Zhang 2015; Yang et al. 2015). Pectin is also used in cosmetic preparations such as cream and lotion as emulsifying or thickening agent (Noreen et al. 2017).

2.2.6. Nutraceutical Potential of Pectin

In recent years, pectin has been shown to have many positive effects on health such as reducing the risk of gastrointestinal health, cancer and cardiovascular disease, regulating glucose tolerance, inhibiting lipase activity and weight control (Wicker et al. 2014). Moreover, there are also studies on pectin that used in drug transport, gene transfer, wound healing and in tissue engineering (Munarin, Tanzi, and Petrini 2012). The functionality of the pectin is a result of the physical effects of the complex components and conformations itself. The potential of pectin extracted for food and pharmaceutical applications varies (Willats, Knox, and Mikkelsen 2006). Therefore, extraction pectins from different sources and characterization has turned into a field of study with intensive research. The most important health effect of natural pectin is due to the fact that it is a soluble dietary fiber. Traditionally, dietary fiber refers to compounds such as plant polysaccharides and lignin that cannot be degraded by human digestive enzymes. Basically, the dietary fibers are divided into two groups: (1) soluble viscous or fermentable fibers (such as pectin), and (2) non-soluble volumetric effect but limited in the fermentable fibers (such as wheat bran). Although the daily recommended consumption of dietary fiber varies according to age, gender or energy needs, it is reported that the average intake of 25 g/2000 kcal dietary fiber is beneficial (FDA, 2016). Pectin is one of the best sources to meet daily dietary fiber needs. Since it cannot be digested, it has been reported that pectin has many physiological effects such as reducing glucose absorption, increasing hypocolestrolemia effect, delaying gastric emptying (Ho, Lin, and Wu 2017). Pectin changes in the intestinal transit time due to gel formation and water holding properties (Gardner et al. 1984). It is known that intestinal mucous secretion is important in the removal of foreign substances. Pectin showed to have positive effect on the formation of intestinal mucosa secretion (Hagesaether and Sande 2008). It has also been reported that pectin reduces the likelihood of developing pathogenic bacteria in the colon and promotes and regulates intestinal microflora. In fact, in one study, it was determined that pectic oligosaccharides inhibited the invasion of the pathogen *Campylobacter jejuni* on Caco-2 cell line (Ganan et al. 2010). In another study, pectic oligosaccharides inhibited the growth of harmful colon bacteria, while *Bifidobacteria* spp.

and *Lactobacillus* spp. have been shown to support the reproduction of species (Parkar et al. 2010). In addition, the prebiotic effect of enzymatic hydrolysate of citrus pectin on Bifidobacterium bifidum and *Lactobacillus acidophilus* was investigated, and a higher increase was observed in the probiotic population than inulin (Ho, Lin, and Wu 2017). In addition, dietary fiber intake reduces the risk of developing certain diseases such as coronary heart disease, stroke, hypertension, diabetes, obesity and gastrointestinal system disorders. Furthermore, an increase in dietary fiber consumption regulates serum lipid concentration, reduces blood pressure, facilitates blood sugar control in diabetes, promotes weight loss and strengthens the immune system (Anderson et al. 2009; Zhang, Xu, and Zhang 2015). It has been observed that increasing dietary fiber consumption significantly improves glycemic control and decreases the need for drugs and insulin in individuals with type 1 or type 2 diabetes (Anderson 1987). Apart from these, pectin has anticarcinogenic effects. In one study, citrus pectins were observed to inhibit proliferation of two human colon carcinoma (HuCC and HT-29) and human leukemia (K562) cell line (Bergman et al. 2010). Beetroot and citrus pectin were modified by the effects of heat and pH, resulting in increased activity of inhibiting metastasis on HT29 and DLD1 colon cancer cells (Maxwell et al. 2016). As can be seen, pectins obtained from different sources have many protective and supportive effects on human health such as prebiotic, cholesterol lowering, preventive of heart diseases, antihypertensive, antidiabetic and anticancer.

CHAPTER 3

OPTIMIZATION OF PECTIN EXTRACTION AND CHARACTERIZATION OF MOLECULAR PROPERTIES OF FIG PECTIN

3.1. Introduction

Pectin extracted primarily from citrus peels and apple pomace is an indispensable ingredient for the food, biomedical, drug, cosmetics, and nutraceuticals industries, not only due to its techno-functional properties but also owing to health-promoting effects as soluble fiber (Gilani et al. 2008; Muñoz-Almagro, Montilla, and Villamiel 2021; Noreen et al. 2017; Rezvanain et al. 2017; Yang et al. 2015). The molecular architecture and functional properties of pectin from different sources are unique. Thus, studies for extraction of alternative pectins from different fruits and their agro-industrial wastes (e.g., from pomelo, berries, hawthorn, sunflower heads, pomegranate peel, cocoa husk, sugar beet pulp, tomato, carrot pomace, pumpkin waste, passion and banana fruit peels, and watermelon rind, etc.) and characterization of their functional properties have become a popular research topic (Dranca and Oroian 2018; Gamonpilas et al. 2021; Henao-Díaz et al. 2021; Li et al. 2021; Marić et al. 2018; Muñoz-Almagro et al. 2021; Reichembach and Petkowicz 2021).

The sun-dried figs have long been known for their rich soluble dietary fiber content that is formed mainly by pectin (Trad, Ginies, et al. 2014a). Due to their high dietary fiber content and bioactive phenolic constituents, fig fruits are historically used as a natural laxative and have been considered as a functional food having positive health benefits on gastrointestinal disorders (Simmons and Preedy 2016; Rtibi et al. 2018). Turkey, with its 85,500 metric tons of production in the 2020/21 season, is the largest producer and exporter of sun-dried figs in the world (Anonymous 2021). Sun-drying is an ancient process to dry fruits that highly affected by the climate and field practices, thus, causes a great variation in the quality of fruits classified as extra quality, class I and class II (UNECE, 2016). The standard sun-dried fruits with 18-22% moisture content are brought to factory and fumigated, washed, examined (for fungal decay and metabolites) and kept in cold storage until packaging. A considerable part of the fruits is also separated as substandard and those seriously damaged by insects, rotting, sun-scalding, split and torn, or excessive drying are used for production of marmalade, molasses, animal feed, or ethanol, depending on their quality and severity of their defects. Recently, a new and rapidly developing trend in the processing of sun-dried figs is that the extra quality sun-dried fruits rehydrated to intermediate moisture levels (~35%) are portion packed, and then they are pasteurized to obtain ready-to-eat, soft, and juicy shelf-stable fruits. The stalks of these premium fruits processed by this emerging method are cut and removed manually before processing. These fig stalks contain part of the flesh tissue that changes between 1 and 1.5% of total fruit weight depending on the experience of the workers employed in stalk cutting. Thus, there is an increased interest by the industry to valorize stalk wastes to produce value-added products. Therefore, extraction and characterization of pectin from processing wastes of fresh or sun-dried figs have recently attracted considerable interest from different researchers. For example, Gharibzahedi, Smith & Guo (2019a, 2019b) extracted and characterized the molecular and functional properties of pectin from peels of fresh figs. In this chapter, fig pectins from sun-dried fig stalk and low-grade (substandard) dried figs have been extracted and optimum temperature, time and acid concentration were determined to obtain the highest pectin yield from the fig wastes. The molecular properties of these fig pectins were also characterized. Besides, some of the results presented in this chapter were already published by Çavdaroğlu, Farris, and Yemenicioğlu (2020), Çavdaroğlu and Yemenicioğlu (2022), Çavdaroğlu et al. (2023).

3.2. Materials

The samples used in extraction optimization part of the study were purees of sun-dried fig processing wastes (a mixture of highly defected fruits, fruit residues from processing, fruits routinely separated for quality control). The cut stalk waste (contains stalk and a piece of fruit flesh that accounts for 1–1.5% of total fruit flesh weight) of high-quality sun-dried figs (Cultivar Sarılop, UNECE class I, size number 9 and 10) separated during processing (fig stalk), and the low quality (UNECE substandard) sun-dried figs (Cultivar Sarılop) which are mainly processed into pastes were used in molecular characterization part of the study. These samples were kindly supplied by KFC Gıda A.S. (İzmir, Turkey) and divided into small portions (one for each kind of raw material) and kept at -20 ^{o}C until use. All samples used were passed from UV inspection for luminescence (free from mycotoxins). Citric acid, citrus pectin (P9135, 79% GA), D-galacturonic acid, 3-phenylphenol, $CaCl_2.2H_2O$, glucose enzyme kit (GAGO20) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Apple pectin was obtained from Tito (Turkey). Sodium hydroxide, hydrochloric acid (37%), sulfuric acid (96%) were obtained from Merck (Germany). Sodium tetrahydroborate was obtained from Carlo-Erba (Milan, Italy). The ethanol used was of technical purity (Tekkim, Bursa). The enzymatic kits for L-arabinose (L-Ara) and D-galactose (D-Gal), and L-rhamnose (L-Rha) (K-ARGA and K-RHAMNOSE) were purchased from Megazyme (Ireland).

3.3. Methods

The methods given below were performed to extract and characterize the molecular properties of fig pectins compared to those of commercial pectins.

3.3.1. Hot Acidic Extraction

Optimization of pectin extraction studies were carried out according to the study of Yuliarti et al. (2015) with small modifications as shown in Figure 3.1. About 50 g of fig sample were blended with 150 mL (1:3, w/v, solid-liquid ratio, SLR) of a solution of citric acid (CA) at different concentrations (1, 3 or 6%, (w/v)) separately for one minute using a laboratory blender (31BL91, Waring Commercial, Torrington, CT, USA) at high speed. The mixture was transferred to a glass beaker and heated at 25, 50 or 95 °C using a magnetic stirrer for 1 or 3 h. The mixture was then centrifuged at 22,668×g, 4 °C for 20 minutes after cooling to 20 °C. After the supernatant was removed (supernatant-1), the residual solid was extracted once again to collect the residual pectin remaining. Then, again on the collected sediment, 1:1 SLR of citric acid solution (1.0%, 3.0% or 6.0%, w/v) was added and heated at 95 °C for 30 minutes. The supernatant was then recovered by centrifugation (supernatant-2) and combined with the previous supernatant (supernatant-1 + supernatant-2) and precipitated with pure ethanol. The mixture of ethanol and extract was stirred at room temperature for 30 minutes and kept at 4 °C overnight without stirring. The precipitated pectin was separated by centrifugation at 22,668×g for 10 minutes and washed twice with pure ethanol. The obtained pectin was collected in a glass petri and allowed to dry for 12-18 h in an oven at 40 °C. For comparative purposes with ultrasound and enzymatic extraction conditions, the acidic extraction was also performed at 1:15 SLR.

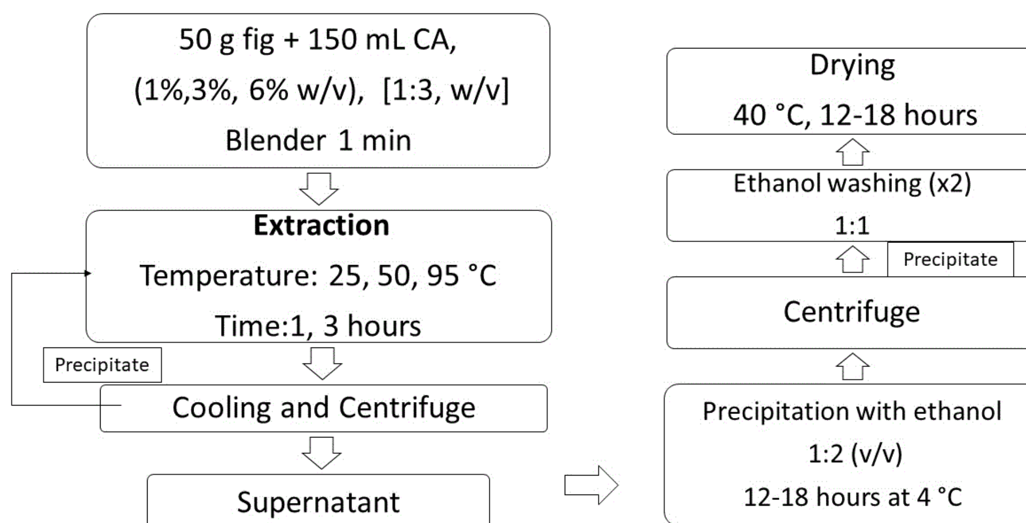

Figure 3.1. Flow diagram of pectin production.

The optimum conditions determined by the procedure described above (6.0% (w/v) CA solution at 95 °C for 1 h) was applied to obtain crude pectin from low-grade dried fig fruits or stalk waste. The pectin in the extract was precipitated with pure ethanol (pectin extract: ethanol ratio = 1:2, v/v) and collected by centrifugation (22,668×g for 10 min). The crude pectins (CFP and CSP) were obtained by drying the collected precipitates for 12-18 h at 40 °C. The pectin acid-extracted from the CSP was further processed to obtain purified fig stalk pectin (PSP) by dissolving in distilled water (1:1, w/w) and pure ethanol (99%, 1:1, w/v) twice before the drying step as shown in Figure 3.2. Further, it was centrifuged at 22,668×g for 10 min at 4 °C and the pellet was recovered and dried for 12-18 h at 40 °C.

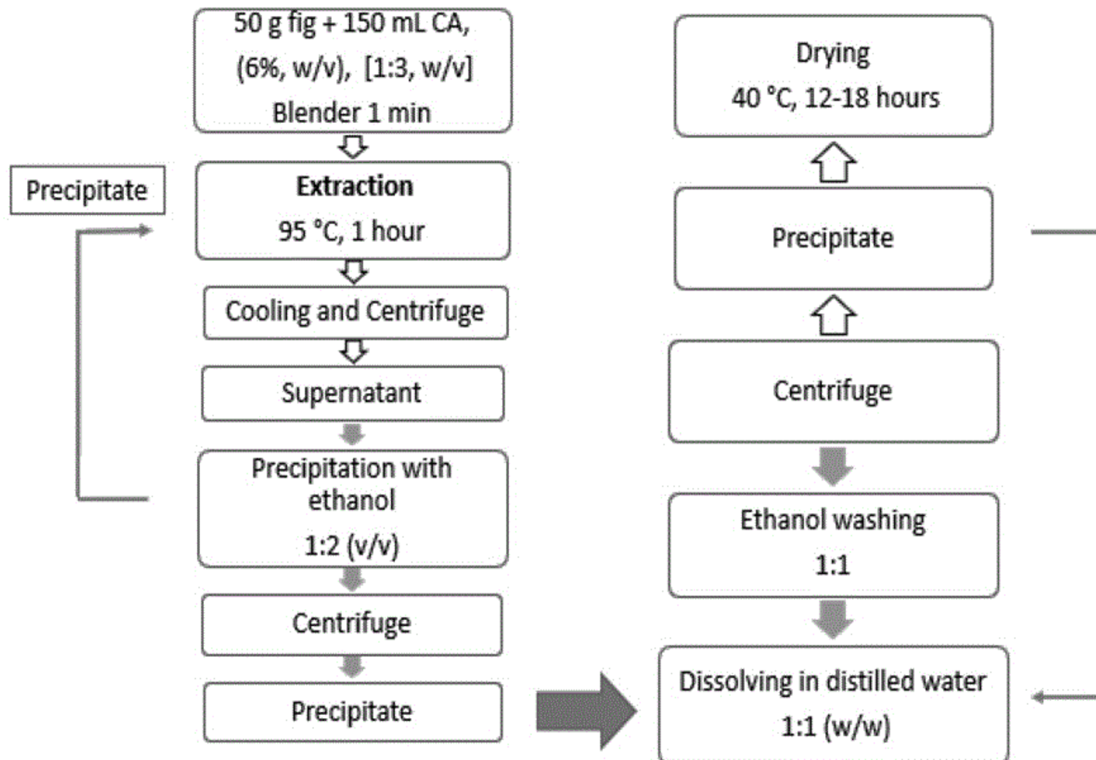

Figure 3.2. Purification of pectin after acid extraction.

3.3.2. Ultrasound-assisted Extraction

To investigate the effect of ultrasound application on the yield was determined by using the method used by Yang et al. (2018). Ultrasound treatment was carried out using a probe ultrasonic processor (Sonics, VC505, USA). The probe ultrasonic processor has a maximum power of 500 W, operated at a frequency of 20 kHz, and the horn microtip has a diameter of 13 mm. The fig sample was mixed and blended with 6.0% (w/v) CA solution. The probe was dipped into the liquid of about 2.5 cm. During sonication, the amplitude was fixed to 100%. Ultrasonic extraction was carried at 1:15 SLR and different time (20, 40 min and 1 h). The temperature of the mixture was increased from 25 °C to 85 °C after 20 min extraction time. Therefore, both the mixture and the device were

cooled down every 20 min. Isolation, precipitation and drying steps of fig pectin were similar to those described in Section 3.3.1.

3.3.3. Enzyme-assisted Extraction

Enzymatic extraction was the other alternative extraction method used to increase pectin yield from fig samples. For this purpose, the methods used by Yang et al. (2018) and Wikiera et al. (2015) were applied with modifications. Celluclast 1.5L (Novozymes, Copenhagen, Denmark) was used as an enzyme with a declared activity of 700 EGU/g which stable at pH 4 - 9. The conditions of enzymatic extraction were 1:15 SLR, different enzyme concentrations of 0.4, 3 and 6% (v/v), 50 °C and pH 5.0 (sodium acetate buffer, 50 mM), and constant shaking (200 rpm) for 18 h. Deactivation of the enzyme was performed by keeping the mixture at boiling water bath for 5 min. Isolation, precipitation and drying steps of fig pectin were similar to those described in Section 3.3.1.

3.3.4. Characterization of Molecular Properties of Fig Pectins and Commercial Pectins

In the following sections, methods performed for determination of molecular properties of fig pectins and commercial pectins are given in detail.

3.3.4.1. Determination of Galacturonic Acid Content

The galacturonic acid content was estimated by the meta-hydroxydiphenyl method (Blumenkrantz and Asboe-Hansen 1973). About 50 mg of fig pectin was dissolved by adding 10 mL of distilled water at 40 ºC. Then, 300 µL of 1:20 diluted pectin solution was taken into a 10 mL screw cap glass tube and a concentrated sulfuric acid solution containing 125 mM sodium tetraborate was added (1.8 mL) and vortexed for 5 seconds. The mixture was then kept in the boiling water bath for 10 min. After cooling the tube in an ice bath, 30 µL of 0.15% m-hydroxydiphenyl prepared in 0.5% (w/v) NaOH was added and waited for 5 minutes. A blank sample was prepared with the addition of 30 µL of 0.5% (w/v) NaOH. The absorbance was read at 520 nm on the spectrophotometer. The standard curve was obtained using standard galacturonic acid solutions at different concentrations (0-100 µg/mL).

Also, GA content of some pectins was analyzed with High Performance Liquid Chromatography (HPLC) by using the Perkin Elmer Series 200 HPLC system with auto-injector (20 µL), column oven, refractive index detector (RID) and Aminex HPX-87H (1,300×7.8 mm, 9µm) column according to the Rumpunen et al (2002) with minor modifications. The enzymatic degradation was applied to 10 mg of pectins by adding 1 mL of 2% (v/v) commercial enzyme Pectinex Ultra SP-L (Novozyme, Denmark) in water. The test tubes were then shaken at 240 rpm for 3 h using an orbital shaker (IKA, OS 5 basic, Germany) placed in an incubator working at 48 ºC. The serial concentrations of pectin (0.5 to 20 g/L) were degraded by Pectinex Ultra SP-L and used for standard curve with drawing galacturonic acid area versus pectin amount. Duplicate measurements were used to calculate the results as % (g GA/100 g of pectin). The flow rate was isocratic at 0.6 mL/min with 5 mM H_2SO_4 as mobile phase at 65 ºC column temperature was used.

3.3.4.2. Determination of Degree of Esterification

The degree of esterification of the fig pectin was determined by the titrimetric method described by Nazaruddin et al. (2013) with small modifications. Initially, 100 mg of pectin was moistened with 0.4 mL of ethanol (96%, v/v). Twenty milliliters of deionized water at 40 ^oC and six drops of phenol red indicator were added and completely dissolved. After that, the mixture was carefully titrated with 0.1 N NaOH until the color changed to fainted pink (V_1). Then, 5 mL of 0.25 N NaOH was added to the solution and shaken vigorously. The solution was allowed to stand for 30 minutes at room temperature. At the end of the period, 5 mL of 0.25 N HCl was added, and the mixture was titrated again with 0.1 N NaOH until the color changed (V_2). The following Equation (2) was used to calculate the degree of esterification (DE):

$$\text{DE\%} = 100\left(\frac{V_2}{V_1+V_2}\right)$$

3.3.4.3. Determination of Degree of Methylation and Acetylation by HPLC

The degree of methylation and acetylation of the pectins were determined according to the method described by Voragen, Schols, & Pilnik (1986) with slight modifications. 30 mg of pectin was suspended in a 1 mL 0.25 M NaOH solution and held at ambient temperature for 2 h. The suspensions were then centrifuged at 10,000×g at 4 °C for 10 minutes and 20 µL of the clear supernatant was injected on the HPLC column (Aminex HPX-87H, Biorad, 300 x 7.8 mm). The column was operated at 35 °C and a

flow rate of 0.6 mL/min with 5 mM H_2SO_4 as mobile phase. Components eluting from the column were detected with a refractive index detector. Experiments were performed in duplicate. The degree of methylation (DM) and acetylation (DA) were expressed as the percent molar ratio of methanol or acetic acid to GA, respectively, using GA quantified by the spectrophotometric method.

3.3.4.4. Determination of Sugar Composition and Molar Ratios

Some sugars such as D-glucose, and L-arabinose, D-galactose, and L-rhamnose used to evaluate the molecular composition of pectin (HG and RG-I ratios and contents) were determined spectrophotometrically using enzymatic kits according to the manufacturer instructions in duplicate samples (Gawkowska et al. 2019). Briefly, the pectin samples (100 mg) were first hydrolyzed chemically with 1.3 M HCL (5 mL) in a sealed glass tube at 100 °C for 1 h. After the tubes were cooled to room temperature, they were neutralized by adding 1.3 M NaOH and adjusted to 100 mL with distilled water. After centrifugation at 10,000 rpm for 10 min, the samples were ready for analysis.

The sugar molar ratios (R-1, R-2, R-3, R-4), homogalacturonan (HG) and rhamnogalacturonan-I (RG-I) contents of the pectins were calculated according to M'Sakni et al. (2006).

HG (mol%)=GA (%)-Rha (%)

RG I (mol%)=[GA (%)-HG (%)]+Rha (%)+Gal (%)+Ara (%)

R-1 (mol%, linearity of pectin) = GA (mol%)/(Rha (mol%) +Ara (mol %) +Gal (mol%));

R-2 (mol%, RG-I fraction content of pectin) = Rha (mol%)/GA (mol%)

R-3 (mol%, degree of branching of RG-I) = (Ara (mol%) + Gal (mol%))/Rha (mol%)

R-4 (mol%, length of Gal branching in RG- I) = Gal (mol%)/Rha (mol%)

3.3.4.5. Determination of Molecular Weight

The weight average molecular weight (Mw), number weighted molecular weight (Mn), and polydispersity index (Mw/Mn) of the extracted fig pectin and commercial citrus pectin were determined by high-performance gel permeation chromatography (HPGPC, Viscotek TDA 302) found in Yıldız Technical University Department of Bioengineering. A sample solution 100 μL was injected into a TSK-Gel G3000PWxl column (Tosoh, Tokyo, Japan). The column temperature was 22 ^{o}C, flow rate was 0.8 mL/min, and mobile phase was 50 mM phosphate buffer containing 0.15 M NaCl (pH 6-7). Viscotek TDA 302 detector system with refractive index (660 nm) and right-angle light scattering (670 nm) detectors were used for on-line SEC signal detection. OmniSEC software was used for the acquisition and analysis of SEC data. Experiments were repeated 2 times for each sample.

3.3.4.6. Ash, Moisture and Protein Contents

The protein contents in pectin samples were determined according to the Bradford method (Bradford 1976). The standard curve was obtained by preparing standard bovine serum albumin (BSA) solutions at different concentrations (0-400 μg/mL). The moisture and ash content of pectin samples were determined according to AOAC (1990) standard method.

3.3.4.7. Fourier Transform Infrared Spectroscopy Analysis

Fourier transform infrared (FT-IR) spectra of pectin samples were collected on a Perkin Elmer FTIR spectrometer (Perkin Elmer – Spectrum 100). The pectin samples were mixed with KBr at a ratio of 1:100. Spectra were collected as an average of 32 scans in the range 4000–450 cm^{-1} with a resolution of 4 cm^{-1} (Jafari et al. 2017). The background was taken under the same conditions with air.

3.3.4.8. Color Analysis

Color measurements were determined using the Minolta CR-400 colorimeter (Minolta Sensing, Osaka, Japan) by recording the L *, a *, b * values.

3.3.5. Statistical Analysis

Statistical difference between treatments was determined by using variance analysis (one way-ANOVA) and Fisher post-test ($p \leq 0.05$) using Minitab (ver.18.1, Minitab Inc., United Kingdom).

3.4. Result and Discussion

The detailed results and discussion will be given in the following sections.

3.4.1. Determination of Acid Extraction Parameters

Generally, the extraction yield of pectin from plant materials varies with the processing conditions as temperature, time, acid type, pH, and solid/liquid ratio (Methacanon, Krongsin, and Gamonpilas 2014). Hence, the first step of this study was focused on finding optimum temperature, time and acid concentration to achieve the highest yield from fig samples. The fig puree was the first sample for the determination of optimum condition. The yield of pectin was in ranged between 4.98% and 9.12% and increased with the increase of CA concentration and extraction temperature (Table 3.1). It is evident that pectin extraction at 95 °C gave a significantly higher yield between 6.7 to 9.1% than those at 25 °C or 50 °C when either 1-h or 3-h extraction was used ($p \leq 0.05$). When the pectin was extracted with 6% CA concentration, the yield was 1.3-fold higher than 3% or 1% CA concentrations at 95 °C. There were no significant differences among yields at 3% or 1% CA concentrations ($p > 0.05$). In contrast, the increase of extraction time from 1 to 3 h did not cause a significant increase in the pectin yield ($p > 0.05$). Thus, the application of 3 h extraction was not beneficial to improve extraction yield. Commercially, pectins are produced from apple, lemon and orange pomaces treated with inorganic acids such as phosphoric, sulfuric and hydrochloric acid at 60-100 ºC between 50-120 minutes (Panouillé, Thibault, and Bonnin 2006). Our conditions were found similar to commercial production. However, in our study organic citric acid was used instead of inorganic acid due to give less damage to the environment. Moreover, in literature, it was found that citric acid had higher chelating ability than other acids. This

led to more interactions with pectin in the plant material and then increased the extraction yield (Yang, Mu, & Ma, 2018).

The basic molecular properties, GA and DE, of fig pectin ranged between 10.1%–25.5% and 57.4%–81.5% depending on the severity of extraction, respectively. The most effective factor on the molecular properties was CA that caused a concentration-dependent reduction in GA and DE of pectin. The increase of the extraction period from 1 to 3 h at a certain concentration of CA had no significant effect on GA of pectin ($p > 0.05$). The increase of the extraction period did not also affect DE in presence of 1% and 3% CA while this caused a significant reduction in DE in the presence of CA at 6% ($p \leq 0.05$). Pectin shows high stability at a pH of 3.5, which corresponds to its pKa value. The degradation of pectin during thermal processing occurs through acid hydrolysis of α-(1-4) glycosidic bonds when the pH drops below 3.0 (Kermani et al. 2014). Under acidic conditions, this degradation process is facilitated by several factors, including a high content of methoxy ester, increasing temperature, rising pH levels, and the presence of monovalent salts, phytates, malates, and citrates (Sila et al 2009). Thus, it seems that combination of increased acid concentration and temperature could enhance pectin hydrolysis. Additionally, more severe treatments could potentially extract pectins that are bound more tightly and may have a lower DE similar to pectin extraction from pomegranate peels with citric acid (88 ºC, 120 min, pH 2.5 with 8% yield) (Pereira et al 2016). The overall results suggested that extraction at 95 °C with 3% or 6% CA for 1 h could be employed to obtain fig pectin yield between 8.0% and 9.0%. Considering the requirements for commercial pectins (higher yield), 95 °C, 6% CA, 1 h acid extraction was chosen for further testing as optimum extraction conditions.

In the literature, studies related to pectin extraction from sun-dried fig processing wastes are scarce. However, the extraction yield obtained at 95 °C with 3% or 6% CA for 1h from sun-dried fig waste in the current work was higher than the pectin yield of 6% obtained by Garibzahedi et al. (2019), who extracted peels of fresh figs with CA-acidified hot water at 90 °C for 1h. On the other hand, the extraction yield of Garibzahedi et al. (2019) from ultrasound-microwave assisted extraction (11.7%) was higher than those obtained in the current work with hot acidic extraction. These results clearly showed that the processing wastes of fresh or dried figs could be evaluated as a source of pectin.

3.4.2. Effect of Extraction Methods on Pectin Yield and Properties

In this thesis, pectin production was achieved by using the commercially widely used hot acid extraction method but using organic acid instead of mineral acid. This acid extraction process optimized in detail previously. Although acidic extraction is highly adopted by the industry due to its economic and efficient features, ultrasonic and enzymatic extraction methods are also gaining importance to produce pectin. Thus, compared with the conventional acid extraction method, enzymatic and ultrasonic extractions were also performed to produce fig pectin in this study.

Table 3.1. Yield, galacturonic acid content and degree of esterification of pectins obtained from fig puree at different process conditions.

Process conditions			Yield	GA	DE
Time (h)	**Temperature (°C)**	**Citric acid (%, w/v)**	**(%)**	**(%)**	**(%)**
1	**25**	**1**	6.11 ± 0.47^{fgh}	20.4 ± 1.67^{cdef}	79.2 ± 0.92^{ab}
		3	5.71 ± 0.44^{hi}	18.4 ± 2.60^{efg}	72.0 ± 2.13^{de}
		6	6.83 ± 0.62^{defg}	13.3 ± 1.99^{hi}	62.7 ± 1.24^{hi}
	50	**1**	5.92 ± 0.51^{ghi}	25.5 ± 2.54^{a}	81.5 ± 1.30^{a}
		3	5.75 ± 1.58^{hi}	21.9 ± 3.55^{abcde}	74.6 ± 1.40^{cd}
		6	7.19 ± 0.33^{de}	16.8 ± 2.19^{fgh}	65.0 ± 2.18^{gh}
	95	**1**	7.00 ± 0.14^{def}	24.6 ± 2.43^{ab}	76.5 ± 2.68^{bc}
		3	8.21 ± 0.35^{abc}	20.2 ± 1.51^{defg}	67.1 ± 0.42^{fg}
		6	9.12 ± 0.49^{a}	18.6 ± 2.94^{efg}	60.8 ± 2.23^{i}
3	**25**	**1**	5.55 ± 0.40^{hi}	21.6 ± 1.94^{bcde}	80.8 ± 2.82^{a}
		3	4.98 ± 0.45^{i}	18.9 ± 3.73^{efg}	74.2 ± 1.22^{cde}
		6	6.11 ± 0.27^{fgh}	16.5 ± 0.95^{gh}	66.2 ± 2.03^{fg}
	50	**1**	5.94 ± 0.44^{gh}	19.5 ± 3.72^{defg}	80.2 ± 2.42^{a}
		3	5.31 ± 0.43^{hi}	18.9 ± 1.01^{efg}	71.4 ± 1.30^{e}
		6	7.51 ± 0.40^{cde}	10.1 ± 1.43^{i}	62.2 ± 0.15^{hi}
	95	**1**	6.70 ± 0.62^{efg}	24.2 ± 0.77^{abc}	79.3 ± 1.68^{ab}
		3	7.74 ± 0.40^{bcd}	22.9 ± 1.50^{abcd}	68.0 ± 1.29^{f}
		6	8.58 ± 0.64^{ab}	18.9 ± 1.19^{efg}	57.4 ± 1.26^{j}

*Data are shown as mean ± standard deviation of triplicate measurements. Data at each column indicated by different letters are significantly different ($p \leq 0.05$).

3.4.2. Effect of Ultrasound-assisted Extraction

Sound waves in the frequency of 20 kHz create cavitation bubbles and disruption of these bubbles induces localized high pressure and increase the temperature of the sample. This temporary cavitation process causes disruption of the cell wall and eases the penetration of the solvent to accelerate the mass transfer of pectin from plant material. By this way, ultrasonication can decrease the processing time while increasing the extraction yield (Marić et al. 2018). For this purpose, it was first tried to apply the SLR of 1:3 as used in acidic extraction, but it was observed that ultrasonication remained ineffective and local at this ratio and could not create a significant movement and bubble density in the sample. Then, it was observed that the effect of ultrasonication increased partially by increasing the SLR to 1:6 and it started to move at a certain speed and homogeneously at the beginning. During processing, the temperature of the mixture increased from 25 °C to 85 °C within 15 min. Evaporation also occurred and the more viscous mixture was obtained again. Homogeneity within mixture was not properly achieved and this would reduce the extraction yield. Hence, the SLR was increased to 1:15 for ultrasonic extraction that gave an effective movement to the sample. To investigate the effect of ultrasound on extraction yield, optimum condition (95 °C, 6% CA) was applied to fig sample using 1:15 SLR and processing time was chosen as an experimental factor. Previously, the linear relation was found between the ultrasonic amplitude and the output power (Xu et al. 2014). Therefore, to give maximum power to the mixture during extraction, the maximum amplitude was chosen as 100%. Accordingly, the ultrasonic extraction yield increased slightly over 10% within 20 min and showed an insignificant decrease at 40 min (Table 3.2). Then, the yield increased significantly ($p < 0.05$) and reached just over 12% at the end of 1 h. Similarly, in the study conducted by Garibzahedi et al. (2019), the highest yield was found to be 13.97% after ultrasound-assisted extraction from fresh fig skins. There was no statistically significant effect of ultrasonication time of 20, 40 or 60 min on the GA and DE values of pectin obtained from low quality dried figs ($p > 0.05$).

Table 3.2. Yield, galacturonic acid content and degree of esterification of pectins obtained from low grade dried fig using ultrasound-assisted extraction.

Time (min)	**Yield (%)**	**GA (%)**	**DE (%)**
20	10.5 ± 0.83[b]	25.8 ± 0.02[a]	45.8 ± 2.87[a]
40	9.59 ± 0.35[b]	28.0 ± 3.04[a]	32.3 ± 1.95[a]
60	12.4 ± 0.33[a]	27.2 ± 0.94[a]	36.5 ± 9.37[a]

*Data are shown as mean ± standard deviation of triplicate measurements. Data at each column indicated by different letters are significantly different ($p \leq 0.05$).

3.4.3. Effect of Enzyme-assisted Extraction

Another method that has recently been used to break down the cellulosic structure of the plant cell wall and to extract many valuable components including pectin is the use of cellulase enzymes during extraction. In this study, frequently used Celluclast 1.5 L enzyme preparation was used to disrupt the cellulose network and facilitate the passage of pectin to the extraction medium (Jeong et al. 2014; Sabater et al. 2018; Wikiera et al. 2015; Yang et al. 2018; Yuliarti et al. 2011). However, the enzyme application cannot be applied under acidic conditions together with heating as in conventional extraction and ultrasonic extraction. Instead, the process must be carried out at an optimum pH and temperature required for the enzyme complex to work (Marić et al. 2018). For this purpose, the literature was examined, and it was decided to carry out the extraction process at pH 5.0 and 50 °C (Wikiera et al. 2015; Yang et al. 2018). Thus, enzymatic extraction is an environmentally friendly method without high temperature and acid. In order to compare the enzyme extraction yield results with the ultrasonication results, the experiments were carried out by adjusting the fig:solvent (pH 5.0, 50 mM Na-acetate buffer) ratio to 1:15, and the extraction was carried out at 50 °C for 18 h using different

amounts of enzyme. As in many enzymatic extraction studies, the concentration of the enzyme was chosen as below 1% (average 0.4%) and exaggerated over 1% (3% and 6%).

Pectin yields obtained by enzymatic extraction processes containing different amounts of Cellucast 1.5L from low quality dried figs are shown in Table 3.3. Accordingly, changing the enzyme concentration did not significantly affect the pectin yield and the yield value varied between 4.1 and 5.5 %. However, exaggerated increase in enzyme concentration decreased pectin GA but did not affect the DE. It should be noted that such enzyme preparations are often used in applications below 1% and that high concentrations in this study are for experimental purposes only. Although the yield of the extraction process using cellulase was low, the DE value of the pectin obtained was as high as 75-81%. This confirms that fig pectin has a high degree of esterification, but the degree of esterification decreases as a result of factors such as acid and temperature used during extraction. The majority of pectins obtained through industrial production are primarily HMP from apple pomace or citrus peel (Thakur et al. 1997). LMP are typically derived from HMP through de-esterification processes involving acid, alkaline, and/or enzymatic treatments (Reichembach and Petkowicz 2021, Chan et al. 2017). Thus, extracting HMP seemed to be advantageous, since it can be adjusted to the LMP by de-esterification.

In order to compare the efficiency of the ultrasonic and enzymatic method with classical acidic extraction, 1 h extraction process with 6% CA at 95 °C was conducted with 1:15 SLR in a single step (the residue is not extracted for the 2nd time). The comparative results between extractions are shown in Table 3.4. Accordingly, it is clear that the enzymatic extraction process can be used to improve the natural properties (such as the DE value) of pectin rather than increasing the production yield, which is the main objective of this project. Besides, ultrasonic-assisted acidic extraction (temperature reached up to 85 °C during process) yielded a significantly higher yield (26% higher) than conventional acidic extraction at 95 °C. Moreover, GA and DE values of pectins obtained by ultrasonic extraction and classical acidic extraction were not significantly different ($p > 0.05$).

Table 3.3. Yield, galacturonic acid content and degree of esterification of pectins obtained from low grade dried fig using enzyme-assisted extraction.

Enzyme concentration (%, v/v)	**Yield (%)**	**GA (%)**	**DE (%)**
0.4	4.10 ± 1.07[a]	37.07 ± 2.11[a]	75.35 ± 0.85[a]
3	5.45 ± 0.91[a]	21.35 ± 5.53[b]	79.30 ± 4.67[a]
6	4.17 ± 1.42[a]	22.31 ± 2.54[b]	81.97 ± 6.64[a]

*Data are shown as mean ± standard deviation of triplicate measurements. Data at each column indicated by different letters are significantly different ($p \leq 0.05$).

Although ultrasound process has provided a significant increase in yield, the main disadvantage of ultrasonic extraction is that the fig:acid solution ratio must be increased to 1:15, and the process has to be carried out for as long as 1 h with acidic extraction by heating. The main advantage of the application of ultrasonic extraction is the increase in yield by shortening the extraction time. In this study, a certain increase in yield was achieved by ultrasonication, but this was achieved in a very long extraction time. In addition, the increase in extract volume dramatically increased the amount of ethanol to be applied during the precipitation of the pectin after extraction (2-3-fold). It is also clear that the volume increase of the extract will require a very large and powerful ultrasonicator with much larger boilers and plant space during commercial production. These issues are very important in terms of initial investment cost and economy of pilot and industrial-scale applications. In contrast, the acidic extraction process can be successfully carried out with simple heated boilers and mixing devices in each plant and with a limited volume of fig:acid solution (1:3) and an acceptable yield can be achieved. Therefore, factors such as cost, and efficiency were also concerned in this study, and it was decided to perform pectin extraction with classical acidic method and to use this pectin for the rest of the study.

Table 3.4. Yield, galacturonic acid content and degree of esterification of pectins obtained from low grade dried fig using different extraction methods.

Extraction Methods		Yield (%)	GA (%)	DE (%)
Ultrasonic	**100% amp, 1:15 SLR, 6% CA, 1 h**	12.4 ± 0.33[a]	27.2 ± 0.94[a]	36.5 ± 9.37[b]
Enzymatic	**3% enzyme, 1:15 SLR, pH 5, 18 h**	5.45 ± 0.91[c]	21.4 ± 5.53[a]	79.3 ± 4.67[a]
Hot acid	**95 °C, 1:15 SLR, 6% CA, 1 h**	9.15 ± 0.45[b]	28.1 ± 0.71[a]	31.3 ± 0.20[b]

*Data are shown as mean ± standard deviation of triplicate measurements. Data at each column indicated by different letters are significantly different ($p \leq 0.05$).

3.4.4. Comparison of Yields and Pectin Properties of Different Fig Samples Extracted at Optimum Conditions

After optimum conditions were found, these parameters were applied to fig stalk and low-grade dried fig samples to show how the yield and properties could change in different samples.

The pectin yield and GA content from fig stalk were found highest as shown in Table 3.5. No significant difference observed between fig puree and low-grade dried fig in terms of both yield and GA content. However, DE was found highest for fig puree and lowest for low grade dried fig and fig stalk found in between. Therefore, since there was not much difference between the fig puree and low-grade dried fig, the rest of the study was continued with low quality figs instead of fig puree. Crude fig pectin produced from low quality dried fig and were denoted as CFP while crude fig stalk pectin produced from fig stalks and denoted as CSP. Also, due to fig stalk pectin having a potential containing high pectin content, it was further purified and showed as PSP. However, purification decreased the pectin yield from 11.68% to 4.55% in fig stalks. Besides, the yield of CSP is also higher than that reported by Gharibzahedi, Smith & Guo (2019b, 2019a) for fresh

fig peel pectin obtained with hot acidic extraction (Yield: 6%) or microwave-assisted extraction (Yield: 9.26%), but it is lower than that of combined ultrasound-microwave assisted extraction of peels optimized with response surface methodology (Yield: ~14%). However, it should be reminded that the yields reported by Gharibzahedi, Smith & Guo (2019a, 2019b) were for a different fig cultivar and section, and it was in fresh form. So, these three pectins were used for the rest of the study.

Table 3.5. Yield, galacturonic acid content and degree of esterification of pectins obtained from different fig wastes.

Acid Extraction (95 ^{o}C, 6% CA, 1 h)	Yield (%)	GA (%)	DE (%)
Fig Puree	9.12 ± 0.49 ^{b}	18.6 ± 2.94 ^{b}	60.8 ± 2.23 ^{a}
Low Grade Dried Fig	9.38 ± 0.01 ^{b}	19.9 ± 0.04 ^{b}	38.8 ± 0.59 ^{c}
Fig Stalk	11.7 ± 0.23 ^{a}	32.3 ± 1.55 ^{a}	50.1 ± 1.29 ^{b}

*Data are shown as mean ± standard deviation of triplicate measurements. Data at each column indicated by different letters are significantly different ($p \leq 0.05$).

3.4.5. Comparison of Composition and Molecular Properties of Fig Pectins with Commercial Pectins

The composition of pectins obtained from whole low-grade (substandard) sun-dried figs and stalk waste (composed of a stalk and adjacent fruit flesh) separated during processing of high-quality sun-dried figs were compared with those of commercial citrus and apple pectins (Table 3.6). The CFP showed the highest ash (~8.2%) and soluble protein (~10.4%) contents reported to be originated mainly from many tiny seeds (florets) within the fruit flesh (Çavdaroğlu, Farris, and Yemenicioğlu 2020). The high ash content determined for fig fruit pectin is also compatible with the literature that reported figs as a

good source of minerals (Trad, Le Bourvellec, et al. 2014b). The CSP obtained from stalk waste lacked seeds. Thus, it showed a similar composition to AP and CP. The fig stalks have no economic value and are treated currently only as waste while sub-standard figs could be utilized by alternative methods (e.g., processing into paste or ethanol and molasses depending on their quality). Moreover, Çavdaroğlu & Yemenicioğlu (2022) have recently shown superior extraction yield (11.7% for stalk and 9.4% for low-grade fruits) and technological properties (e.g., better emulsion stabilizing capacity and gel textural properties) of fig stalk pectin than fig fruit pectin. Therefore, the purified pectin (PSP) in the current work was obtained using stalk wastes. The CSP and its purified form PSP contained similar ash and soluble protein contents ($p > 0.05$). The soluble protein contents of CSP and PSP were found to be similar with CP, but significantly higher (1.7 and 1.9-fold) than that of AP.

The molecular properties of pectins used in this study are shown in Table 3.6. The GA of pectins varied between 32.2 and 80.4%. The CP showed the highest GA (80.4%) while AP (63.2%) and PSP (63%) had intermediate GAs, and crude pectins, CSP (34.2%), and CFP (32.2%) had the lowest GAs. It is important to note that the GAs determined for crude pectins extracted from sun-dried figs and their stalks were in the range of those (24.5-33.4%) determined by Trad et al. (2014b) for pectic compounds extracted from different fresh Tunisian fig cultivars. In contrast, the purified stalk waste pectin, PSP, showed a 1.8-fold higher GA content than its crude form (CSP). Considering the DE values, the CP, AP and PSP could be classified as high-methoxyl pectins (HMP, DE > 50%), while CSP and CFP are low-methoxyl pectins (LMP, DE < 50%). The increased DE of PSP with purification could be attributed to the increased proportion of high methoxyl GA fractions by insolubilization of LMP fractions during repeated solubilization-alcohol precipitation cycles. The sun-dried figs contain very high pectin methylesterase activity (Demirbüker et al., 2006); thus, a heterogeneity in DE of extracted fig pectins is expected. It is also noteworthy that the GA and DE of PSP are higher than those of fresh fig peel pectin (GA: 52.5%, DE: 39%) obtained with hot acidic extraction by Gharibzahedi, Smith & Guo (2019a). The GA and DM values determined for pectins by the HPLC methods were found similar to GA and DE determined by the spectrophotometric and titrimetric methods, respectively. The DA of pectins used in the current work also showed a great variation between 2.22% and 29.9%. The fig pectins showed significantly higher DA than commercial pectins. The PSP showed the highest

DA. Thus, it appears that the purification eliminated not only LMP fractions but also pectin fractions with low DA. The DE and DA are highly effective in gelation and emulsifying properties of pectins (Broxterman et al. 2017; Schmidt et al. 2015; Vriesmann & Petkowicz, 2013). However, data about the effect of DA on film properties of pectin are scarce. In the literature, the DAs of some pectins were given as follows: 3% for citrus and 14% for pear pectins (Voragen et al. 1986), 18-58% for okra pectins (Sengkhamparn et al. 2009), 17% for cacao pod husk pectin (Vriesmann & Petkowicz, 2013), 20% for carrot pectin (Broxterman et al., 2017), and 16-46.2% for sugar beet pectin (Bindereif et al. 2021).

The sugar molar ratios (R-1, R-2, R-3, and R-4) of pectins were also estimated by determining the amount of their major sugars, D-glucose (D-Glc), L-rhamnose (L-Rha), D-galactose (D-Gal), and L-arabinose (L-Ara) (Çavdaroğlu & Yemenicioğlu, 2022). The D-Glc content of CSP, CFP, and AP did not vary considerably and changed between 5.80 and 7.05%, while CP obtained from citrus peels contained a limited D-Glc content. It is also important to report that the purification caused almost 3.6-fold lower glucose content for PSP than CSP, which is the crude form of PSP. The CP and CFP showed low levels of L-Rha while AP, CSP, and PSP contained significantly higher L-Rha (3.4 to 5.5-fold) than these two pectins ($p \leq 0.05$). The highest D-Gal content was found for PSP (6.3%), followed by slightly to moderately lower D-Gal contents of CFP, CP, and CSP, and considerably lower D-Gal content of AP (1.53%). Finally, the CP, CFP, and CSP contained similar L-Ara contents ($p > 0.05$) changing between 2.96 and 3.59%, while AP and PSP contained significantly lower and higher L-Ara contents than those of other pectins, respectively ($p \leq 0.05$).

In plant cell walls, the pectin is mainly formed by homogalacturonan (HG, ~65%), while rhamnogalacturonan-I (RG-I, ~20-35%) is the second dominant structural form, and rhamnogalacturonan-II (RG-II, ~10%) is the minor fraction (Alba & Kontogiorgos, 2017; Basak & Annapure, 2022; Chandrayan, 2018; Yapo, 2011). Since RG-II is a very complex minor fraction composed of many different sugars, it is not considered in the theoretical calculations (Houben et al. 2011; M'sakni et al. 2006). The R-1 values suggested that the AP and CP showed the highest molecular linearity while PSP had lower-intermediate linearity, and CFP and CSP had the lowest linearity. According to R-2, a ratio that is an indirect indication of RG-I content, the highest value was observed for CSP, followed in descending order by PSP, AP, CFP and CP. The R-3 and R-4 also suggested that the degree of branching and D-Gal branch length of RG-I for pectins in

descending order were as follows: CP, CFP, PSP, CSP, and AP. The calculated HG contents showed some parallelism with GA contents of CP, AP and PSP, but some variations in parallelism were observed between HG and GA of CFP and CSP. The calculated RG-I showed that the CSP and CP are the fractions with the richest and poorest RG-I contents, respectively. This finding showed parallelism with R-2 ratios, which also indicates RG-I content. Both the RG-I and R-2 also indicated that PSP is the second RG-I rich fraction after CSP. However, RG-I contents did not show parallelism with R-2 of AP and CFP, possibly due to the large differences (almost 3-fold) between their L-Rha contents that affect R-2 significantly (L-Rha (mol%)/GA (mol%)).

Table 3.6. Different characteristics of commercial pectins and different fig pectins.

Characteristics*	CP	AP	CFP	CSP	PSP
Compositions of different pectins**					
Moisture content	12.77±0.17[a]	7.76 ± 0.88[c]	9.10±0.29[b]	6.76 ± 0.11[d]	5.06 ± 0.25[e]
Ash	4.19 ± 0.20[b]	4.92 ± 0.62[b]	8.19 ± 0.01[a]	4.61 ± 0.91[b]	4.85 ± 0.37[b]
Soluble protein	5.69 ± 1.04[b]	2.96 ± 0.21[c]	10.4 ± 0.31[a]	4.94 ± 0.09[b]	5.70 ± 0.26[b]
Molecular properties of different pectins**					
GA	80.4 ± 7.90[a]	63.2 ± 0.30[b]	32.2 ± 3.94[c]	34.2 ± 3.73[c]	63.0 ± 4.52[b]
GA-HPLC	77.1 ± 0.21[a]	44.7 ± 0.44[c]	21.3 ± 0.13[e]	30.5 ± 0.08[d]	62.3 ± 1.25[b]
DE	54.6 ± 1.46[c]	60.9 ± 2.05[b]	36.7 ± 3.95[e]	45.0 ± 2.52[d]	65.9 ± 1.89[a]
DM-HPLC	58.0 ± 1.85[bc]	60.4 ± 4.95[ab]	29.8 ± 4.15[d]	53.4 ± 5.67[c]	67.0 ± 5.97[a]
DA	3.16 ± 0.47[d]	2.22 ± 0.08[e]	6.95 ± 0.83[c]	11.6 ± 0.02[b]	29.9 ± 3.82[a]
D-Glc	0.24 ± 0.06[d]	7.05 ± 0.23[a]	5.80 ± 0.02[b]	6.15 ± 0.52[b]	1.72 ± 0.09[c]
L-Rha	0.33 ± 0.09[b]	1.56 ± 0.01[a]	0.46 ±0.05[b]	1.81 ± 0.17[a]	1.66 ± 0.40[a]
D-Gal	3.79 ± 0.42[bc]	1.53 ± 0.02[c]	4.08 ± 0.19[b]	3.60 ± 0.32[b]	6.26 ± 0.73[a]
L-Ara	2.96 ± 0.80[b]	1.79 ± 0.07[c]	3.12 ± 0.14[b]	3.59 ± 0.31[b]	4.92 ± 0.43[a]
R-1***	9.81	10.90	3.59	3.26	4.19
R-2***	0.005	0.029	0.017	0.063	0.031
R-3***	20.96	2.14	15.51	4.03	6.74
R-4***	10.82	0.89	8.08	1.82	3.47
HG***	89.98	80.11	66.63	62.14	76.30
RG-I***	9.72	9.98	20.14	25.00	21.38

*Data are shown as mean ± standard deviation of triplicate measurements. Data at each row indicated by different letters are significantly different ($p \leq 0.05$). GA: galacturonic acid content; DE: degree of esterification; DM: degree of methylation; DA: degree of acetylation; D-Glc: D-glucose; L-Rha: L-rhamnose; D-Gal: D-galactose; L-Ara: L-arabinose; R-1, R-2, R-3, R-4: sugar molar ratios.; HG: The molar percentage of homogalacturonan; RG-I: The molar percentage of rhamnogalacturonan-I.

** Data are expressed as % on a dry basis of pectin powder except moisture content.

*** Values are mol%.

3.4.5.1. Molecular Weight of Fig Pectin and Commercial Pectin

Molecular weight characteristics of CSP and CP were determined by GPC as shown in Table 3.7, but the chromatography of CFP cannot be performed due to intense and stable turbidity of samples caused probably by high protein content (Qiu, Zhao, and McClements 2015). Although the Mw of CSP (19.2×10^4 Da) was significantly higher than CP (13.4×10^4 Da) ($p \leq 0.05$), the Mn values of these two pectins were not found statistically different ($p > 0.05$). In the literature, data about Mw and Mn of sun-dried fig pectin are scarce, but Gharibzahedi, Smith & Guo (2019a) reported that the molecular weight of fresh fig peel pectin extracted by different methods was in the $5.3\text{-}6.9 \times 10^3$ kDa range. The different molecular weight as well as DE and GA of fresh fig peel pectin than fig stalk waste pectin could be due to the differences in extracted fruit sections and/or cultivars (Zhong et al. 2010; Wongkaew et al. 2021). Moreover, the peel pectin studied by Gharibzahedi, Smith & Guo (2019a) was from a fresh peel tissue that might contain highly active pectinases that could affect molecular properties of pectin at pre- and post-extraction stages. The Mw of CSP is also slightly higher than that of 8.8×10^4 Da reported for dragon fruit (Muhammad et al. 2014), but lower than that of $2.4\text{-}16.5 \times 10^5$ Da determined for kiwifruit (Yuliarti et al. 2011). It is also important to note that the Mw of 13.8×10^4 Da reported by Muhammad et al. (2014) was quite similar with Mw for CP determined in the current work. The Mw/Mn ratios of CSP and CP determined in the current work also suggested that both CSP and CP were heterogenous pectins consisting different fractions.

Table 3.7. Comparison of molecular weights of commercial citrus pectin and crude fig stalk pectin.

	Mn - (10^4 Da)	Mw - (10^4 Da)	Mw/Mn
CP	3.27 ± 1.56^a	13.4 ± 1.77^a	4.50 ± 1.61^a
CSP	3.94 ± 0.87^a	19.2 ± 0.87^a	4.98 ± 0.88^a

*Data are shown as mean ± standard deviation of triplicate measurement. Data at each column indicated by different letters are significantly different ($p \leq 0.05$).

3.4.5.2. Comparison of FT-IR Spectra of Fig Pectins and Commercial Pectins

The FT-IR spectra of novel pectins are frequently compared with those of well-known commercial citrus pectin to confirm their molecular compatibility (Jafari et al. 2017; Lira-Ortiz et al. 2014). In general, fig pectins gave identical peaks with commercial pectins as shown in Figure 3.3. The v-shaped band around the region of 3420-3436 cm^{-1} referred to intermolecular and intramolecular OH bonds in the structure of pectins (Gnanasambandam and Proctor 2000). These peaks originate probably from –OH groups of galacturonic acid and other sugar residues in pectin structure and water molecules absorbed by the hygroscopic samples. The peak between 3029 and 2919 cm^{-1} reflected the stretching vibrations of the CH bond belonging to CH, CH_2, and CH_3 groups in these pectins (Sinitsya et al. 2002). The two bands around 1628 cm^{-1} and 1747 cm^{-1} showed the free carboxyl (COO-) and ester-carbonyl (C–O) groups are important since they are used to estimate the degree of esterification and galacturonic acid content of pectins (Fellah et al. 2009; Gnanasambandam and Proctor 2000; Kyomugasho et al. 2015). The CH, C–OH, and α-1,4 glycosidic (COC) bonds in the galacturonic acid chain were also detected at 1384, 1296 and 1198 cm^{-1}, respectively. All pectins studied gave peaks at or closely around these bands with different intensities. It was reported that the band region between 1300 and 800 cm^{-1} of the spectrum is called the fingerprint region that is specific to the material structure and difficult to interpret (Jafari et al. 2017). Therefore, it was

proved that the CFP, CSP, and PSP FT-IR profiles were highly comparable with those of CP and AP.

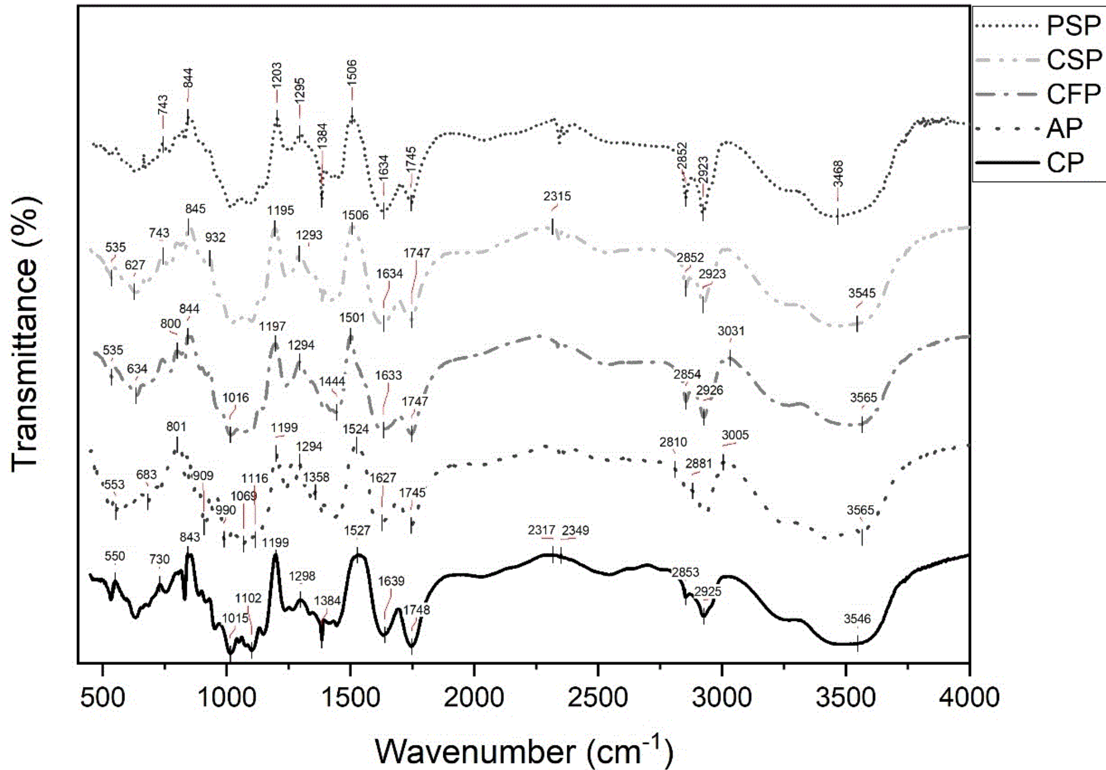

Figure 3.3. FT-IR spectra of commercial pectins and different fig pectins.

3.4.5.3. Color of Fig Pectins and Commercial Pectins

The photographic images of pectins are given in Figure 3.4 and color values are given in Table 3.8. The dried fig pectins (CFP, CSP and PSP) were darker in color than citrus (CP) and apple pectin (AP), which were similar in color. The luminous values (L*) of pectins are varied in a range between 49.4 and 72.7. The CP and AP had significantly higher luminous values (L*) than CFP, CSP and PSP ($p \leq 0.05$). Purification of CSP

didn't change the L* values ($p > 0.05$) whereas the a* and b* values decreased significantly for PSP ($p \leq 0.05$). The redness (a*) values of pectins are changed from 3.93 to 7.58 while the yellowness (b*) values are varied from 11.7 to 22.6. Figs are dried both in the tree and under the sun for a long period of time that can cause the accumulation of Maillard reaction products. These brown-colored products complex with pectin and other polysaccharides resulted in a dark color. However, if necessary, the color of the fig pectin produced can be bleached by various solvents through washing processes. In the case of a* values, the opposite was observed, and the redness values of CFP and PSP were higher than those of commercial pectins and PSP. Conversely, the b * values of CP were significantly higher than other pectins ($p \leq 0.05$).

Figure 3.4. Photographic images of commercial pectins and different fig pectins: [citrus pectin (CP) (A), apple pectin (AP) (B), crude low-grade dried fig pectin (CFP) (C), crude fig stalk pectin (CSP) (D) and purified fig stalk pectin (PSP) (E)].

Table 3.8. Color values of commercial pectins and different fig pectins.

Characteristics	CP	AP	CFP	CSP	PSP
L*	72.3 ± 0.44^{a}	72.7 ± 0.09^{a}	49.4 ± 0.17^{c}	59.3 ± 1.18^{b}	59.8 ± 2.32^{b}
a*	6.20 ± 0.13^{c}	4.87 ± 0.06^{d}	7.58 ± 0.15^{a}	6.86 ± 0.43^{b}	3.93 ± 0.62^{e}
b*	22.6 ± 0.14^{a}	15.5 ± 0.30^{c}	14.9 ± 0.17^{c}	16.8 ± 0.95^{b}	11.7 ± 0.47^{d}

Data are shown as mean ± standard deviation of triplicate measurement. Data at each row indicated by different letters are significantly different ($p \leq 0.05$). L: Lightness, a*: redness/greenness, b*: yellowness/ blueness.

3.5. Conclusions

Based on the presented results, the economically feasible and effective hot acidic extraction method was selected to produce pectin from low-grade dried figs and fig stalk. Both the hot acidic and ultrasound-assisted extractions provided acceptable pectin yields and similar pectin molecular properties (GA and DE), but the former was preferred over the latter since it enables extraction at low SLR, thus giving lower costs during ethanol precipitation of pectin applied during purification. On the other hand, pectin obtained by the enzyme-assisted extraction method was not preferred since this method gave the lowest pectin yield. The pectin obtained by the enzyme-assisted method showed a similar GA content with those of the others, but it gave almost 2-fold higher DE value. This finding clearly proved that the fig pectin shows inherently high DE, but it is deesterified very rapidly due to its high sensitivity to heat and ultrasonication at acidic conditions.

Crude pectins obtained by low-grade dried figs and fig stalks with twice alcohol precipitation showed galacturonic acid content (GA) and esterification degrees (DE) of less than 50% and were classified as low methoxyl pectins. However, further purification of pectin with repeated ethanol precipitation (three times) was found effective in producing high methoxyl fig pectin showing GA and DE values over 50%.

Fig stalk pectin had similar properties to citrus pectin in terms of molecular weight. The protein content of pectin obtained from fig stalk wastes was at the level of citrus and apple pectins, but the pectin obtained from fig fruit contained excessive (10%) protein due to the proteins passed into extract from tiny fruit seeds. The degree of linearity (HG) was found to be significantly high for commercial pectins whereas crude fig fruit pectins show low degree of linearity, and fig stalk pectin showed a moderate degree of linearity. These properties could impact the functionality of the pectin, such as viscosity, gelling, and emulsification, which will be shown in the following chapters of this thesis.

CHAPTER 4

CHARACTERIZATION OF FUNCTIONAL PROPERTIES OF FIG PECTINS

4.1. Introduction

Commercial pectin is employed in the food industry mainly as a gelling, thickening, water-holding, and coating agent for a great variety of foods. It is used as a gelling agent in jams and confectionery; thickening and texturizing agent in yogurt, milk drinks, fruit milk beverages, sauces, and ice cream; and as coating agent in minimally processed fruits and vegetables (Moslemi 2021; Ciriminna et al. 2016; Yemenicioğlu et al. 2020). The FAO/WHO committee on food additives has recommended pectin as a safe additive, without any specific limit on daily intake except adherence to good manufacturing practices (FDA 2023). Although pectin is commonly used as a food additive within the range of 1.5 to 2.0% (w/w), the properties of pectin are also important in determining the amount. The choice of pectin for a particular food product depends on various factors such as desired texture, pH level, processing temperature, presence of ions and proteins, and expected shelf life (Thakur et al. 1997). Pectin is also an important ingredient for biomedical, drug, cosmetics and nutraceuticals industry (Gilani et al. 2008; Rezvanain et al. 2017; Yang et al. 2015; Noreen et al. 2017). Thus, to meet the growing demands of global pectin market, extensive studies have been directed toward to improve methods of pectin extraction (mainly by ultrasonic and enzymatic applications) from

common sources and to find alternative sources of pectin (Cui et al. 2021; Ciriminna et al. 2016; Khedmat et al. 2020; Liu et al. 2020). Sunflower head and sugar beet pulp are emerging sources of pectin since they are important agro-industrial wastes that contain considerable amount of pectin (10 to 30% by dry weight) (Gawkowska, Cybulska, and Zdunek 2018; Dranca and Oroian 2018). There is also growing interest to valorize some other agro-industrial wastes such as tomato, carrot and pumpkin waste, passion and banana fruit peels, and watermelon rinds (Dranca and Oroian 2018). The search for alternative pectin sources is beneficial not only to increase utilization of processing wastes, but also to discover pectin with novel unique functional properties. For example, although gelation capacity of pectin from sugar beet pulp is inferior than that of citrus pectin, it has unique emulsifying properties originating from covalently bound hydrophobic protein that turns hydrophilic pectin into a surface active hydrocolloid (Williams et al. 2005; Pacheco et al. 2019).

The production of fig pectin and characterization of its technological properties and health benefits have attracted interest of different researchers. For example, Gharibzahedi, Smith & Guo (2019a, 2019b) extracted and characterized pectin from peels of fresh figs. Moreover, Çavdaroğlu et al. (2020) extracted pectin from whole sun-dried figs using classical hot acidic extraction with a yield between 8 and 9% and characterized its edible film properties and applicability as a fruit coating. In this chapter, pectin from sun-dried fig stalk waste and whole sun-dried figs have been extracted and characterized for its molecular and functional properties. The functional properties of these fig pectins were also compared with those of commercial citrus and apple pectins to understand their industrial relevance. This work is original in that it is the first study related to the functional properties of pectin from sun-dried fig stalk waste and whole sun-dried figs. Besides, some of the results presented in this chapter were already published by Çavdaroğlu and Yemenicioğlu (2022).

4.2. Materials

The samples used in this part of the study were sun-dried cut stalks together with small amounts of fruit pieces (about 1.5%) of high-quality dried figs before portion packaging ("fig stalk") and the lowest quality dried figs which are mostly processed into the paste ("low grade dried figs"). These samples were kindly supplied by KFC Gıda A.S. (İzmir, Turkey) and divided into small portions (one for each kind of raw material) and kept at -20 ^{o}C until use. All samples used were fluorescence tested (free from mycotoxins).

Citrus pectin (P9135, 79% GA), $CaCl_2.2H_2O$, ABTS (2,20-azino-bis(3-ethylbenz-thiazoline-6-sulfonic acid)), sodium azide were purchased from were obtained from Sigma-Aldrich (St. Louis, MO, USA). Apple pectin was obtained from Tito (Turkey). Tartaric acid, sodium citrate, sodium carbonate, sodium chloride, di-Sodium hydrogen phosphate, sodium dihydrogen phosphate, potassium persulfate were obtained from Merck (Germany). Sodium tetrahydroborate was obtained from Carlo-Erba (Milan, Italy). The sucrose (table sugar) and sunflower oil were obtained from a local supermarket. Trolox (6-hydroxy-2,5,7,8-tetra- methylchroman-2-carboxylic acid) and Folin-Ciocalteu reagent were purchased from Fluka (Switzerland).

4.3. Methods

The methods given below were performed to characterize the functional properties of fig pectins and compared with those of commercial pectins.

4.3.1. Foaming Capacity and Foam Stability

The foaming capacity and stability of the pectin samples were performed according to Aydemir & Yemenicioĝlu (2013). Twenty mL of pectin solution (10 mg/mL) was prepared at room temperature. The solution was then homogenized with ultraturrax at 23,000 rpm for 1 min to induce foaming. The foaming capacity (FC) was determined by measuring the volume of the formed foam as mL. The foam stability (FS) was determined by measuring foam volume at 30th and 180th min.

4.3.2. Emulsifying Capacity and Emulsion Stability

The emulsifying activity of pectin samples and the stability of prepared emulsions will be assessed according to the method described by Raji et al. (2017) with modifications. The determination of emulsifying activity is based on the ratio of the emulsified layer volume and the whole volume of the solution. Emulsions were prepared by adding 5 mL of vegetable oil to 5 mL of pectin solution (1-3%, w/v). The mixtures were homogenized with ultraturrax at 15,000 rpm for 3 min at room temperature. To prevent microbial degradation of emulsions, sodium azide (0.01%, w/v) was added as a preservative during homogenization. The samples were then stored at 25 °C for 2 weeks. The emulsification capacity (EC) was determined by measuring the volume of an emulsion of a prepared solution (mL) after 30 minutes. Emulsion stability (ES, %) was determined by the ratio of the emulsified layer volume (mL, ELV) at the end of incubation times (day 1 and day 7) to the initial volume (mL, Wv) of the emulsion.

$$\text{ES (\%)} = \frac{\text{ELV}}{\text{Wv}}$$

The stability of emulsions at 3% (w/v) pectin was also determined by monitoring the particle size distributions at the beginning and after 7 days at 25 °C using dynamic light scattering (DLS) system (NanoPlus DLS, Micromeritics Instrument Corporation,

GA, USA). The surface mean diameter (D[3,2]) values were recorded. The data were reported as an average of 3 repeated measurements of two replicates.

4.3.3. Determination of Viscosity

The viscosity of 3% (w/v) of pectin solution was determined with a Haake VT 550 Viscometer (Haake MessTechnik Gmbh Co., Karlsruhe, Germany) with a SV-DIN sensor at room temperature (Monsoor 2005).

4.3.4. Determination of Gelling Capacity

The two types (high ester or low ester) of standard pectin gels with different pectin concentrations (0.4-3%, w/w) were prepared according to the Food chemicals codex (1972) in the back extrusion cell of the texture analyzer (TA-XT2, Stable Microsystems, Godalming, UK).

In high ester pectin jelly preparation, 0.22 g of pectin was transferred into the beaker and mixed thoroughly with 2.5 g sucrose. A 20.5 mL of distilled water was added to the beaker and mixed with pectin and sucrose using an ultraturrax for 2 min at 10,000 rpm (Heidolph, Germany, rotor ϕ = 6.6 mm Tip). The mixture was heated and boiled while stirring. A 14.9 g of sucrose was then added to the pectin-sucrose mixture and stirring was continued until a net weight of 50.75 g was reached. The mixture was removed from the heat and allowed to cool for 1 min. By the time, the back-extrusion cell was prepared by adding 0.1 mL of 48.8% (w/v) tartaric acid solution. After that, the solution was immediately poured into the cell while stirring to mix the sample with the

tartaric acid solution. The gel was held for 1 h at room temperature by wrapping with the aluminum foil on top and then stored at 4 ºC for 18-24 h until the testing.

The gel strength of pectin was determined by using texture profile analysis (TPA) with a texture analyzer (TA-XT2, Stable Microsystems, Godalming, UK). Before testing, gels were stored at 25 ºC for 30 min to reach the definite temperature controlled by thermocouple. The gels were deformed by compression at a constant speed of 0.50 mm/s to a distance of 15 mm from the gel surface using a P/1R cylinder delrin radiused plunger (diameter 25.4 mm). Hardness, adhesiveness, springiness, cohesiveness, gumminess, chewiness, and resilience parameters were recorded. Duplicate experimentation was carried out.

4.3.5. Water and Oil Absorption Capacity

The water (WAC) and oil absorption capacity (OAC) of the pectin samples was performed according to Aydemir & Yemenicioĝlu (2013). Fifty mg of pectin sample and 1.5 mL of distilled water or commercial sunflower oil were mixed at room temperature for 20 s by a vortex in a 2 mL centrifuge tube. After mixing, the tubes were incubated at 30 °C for 30 min. The tubes were then centrifuged at 5000×g, 25 °C for 10 min and the separated free water or oil in their supernatants were removed carefully. The absorbed water or oil content was determined by the weighing of the tubes. WAC and OAC were expressed as g of water or oil absorbed per g of pectin, respectively.

4.3.6. Antioxidant Activity

The Trolox equivalent antioxidant capacity (TEAC) method was used for the determination of the free radical scavenging-based antioxidant activity of pectins using ABTS as a free radical according to Re et al (1999). Briefly, 10, 20 and 30 μL of 5 g/L pectin samples were mixed with 2 mL of ABTS free radical cation solution prepared in 150 mM NaCl-0.01 M Na-phosphate buffer at pH 7.4. The reduction in absorbance values was recorded at 734 nm with a spectrophotometer (Shimadzu Model 2450, Japan) for test periods of 1, 3, 6, 9, 12, and 15 min. The Trolox was used as a standard (0-2 mM). The area under the curve (AUC) values of triplicated samples were calculated to find TEAC values, and antioxidant activity was expressed as mmol Trolox equivalents per 100 g of pectin. The analyses were repeated three times for each of the pectin samples given as μmol Trolox/100g. The total phenolic content (TPC) of extracted pectin was determined spectrophotometrically at 760 nm using the Folin-Ciocalteau's reagent as reactive compound and gallic acid (GAE) as a standard (Singleton and Rossi 1965). The average of triplicate measurements was expressed as mg GAE/100 g of pectin.

4.3.7. Statistical Analysis

Statistical difference between treatments was determined by using variance analysis (one way-ANOVA) and Fisher post-test ($p \leq 0.05$) using Minitab (ver.18.1, Minitab Inc., United Kingdom).

4.4. Result and Discussion

The detailed results and discussion will be given in the following sections.

4.4.1. Foaming Capacity and Foam Stability

The pectins are frequently tested for their foaming capacity and stability since they mostly contain complexed surface-active protein fractions which show thickening effects that improve the foam stability (Dickinson 2003). The FC and FS of different pectins are given in Figure 4.1. The AP could not form foam. The reason behind was thought to be its high viscosity. The CP and CSP showed the same FC that was significantly higher (~1.8 mL) than that of FP and PSP ($p \leq 0.05$). In contrast, the CFP showed the highest FS by maintaining 100% its initial foam volume at the end of 30 min while there were no significant differences between CP and PSP at this period ($p > 0.05$). Although there was no significant difference between FS_{30min} values of CP, PSP and CSP ($p > 0.05$), CP maintained its foam stability better than PSP after 180 min (FS_{180min}). Since carbohydrates are not surface-active molecules, their properties that require surface activity such as foaming and emulsion capacity/stability are often attributed to proteins that are ionically or covalently bound at their surface (Wicker et al. 2014). Therefore, the high FS of CFP was possibly associated with its higher protein content than other pectins. Moreover, it is a well-known fact that polysaccharides can contribute to the stability of the foams by increasing the viscosity in the foam-forming environment (Petkowicz, Vriesmann, and Williams 2017).

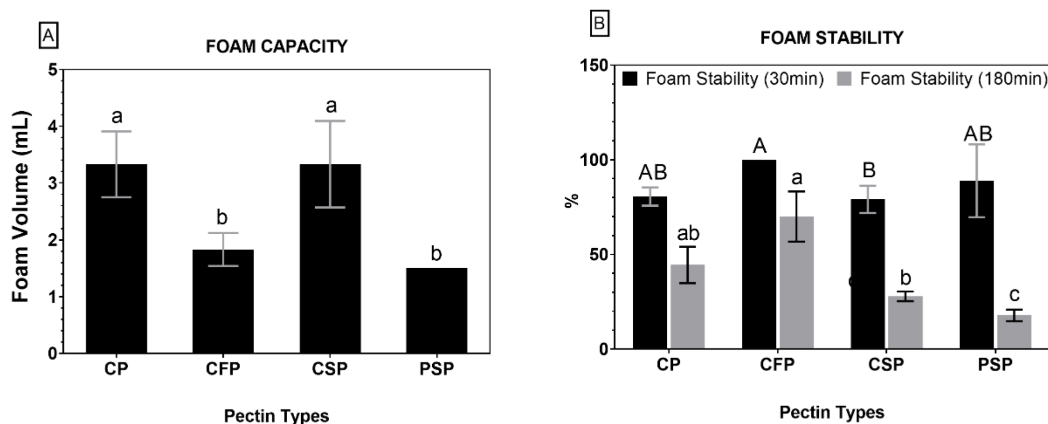

Figure 4.1. Foam capacity (A) and foam stability (B) of commercial citrus and different fig pectins. Each data point is shown as the average of three replicates. The error bar indicates standard deviation. a-c and A-B at each bar denote a statistically significant difference separately ($p \leq 0.05$).

4.4.2. Emulsification Capacity, Emulsion Stability and Particle Size Characteristics

The EC and ES of different pectins between 1 and 3% (w/v) are seen in Figure 4.2. The ECs of all pectins were not significantly different at pectin concentrations of 2 and 3% (w/v, $p > 0.05$) (Fig. 4.2A). However, the reduction of pectin concentration to 1% (w/v) reduced the ECs of CP and CFP more significantly than EC of AP, CSP and PSP that showed similar values at all pectin concentrations ($p > 0.05$). The ESs of pectins at the end of 1- and 7-days also showed some differences (Fig. 4.2B and 4.2C). The CSP and PSP at 3% (w/v) gave significantly higher ES than those at 1 and 2% (w/v) at the end of 1- and 7-days ($p \leq 0.05$). The ES of AP at 3% was also higher than those at 1 and 2% (w/v) at end of 1-day, but the CFP concentration did not affect the ESs at the end of 7-days. The results of 7-days storage showed that the CSP and PSP at 3% (w/v) showed the highest ES value (~96.16%) while PSP at 2% (w/v) was the second highest value

(~82.02%) at the end of 7-days. It is important to note that the CP at 3% (w/v) was the third highest followed by AP and CFP at 3% (w/v) within 7-days. However, there were no significant differences among ESs of CP, AP, CFP and CSP at 1 and 2% (w/v) compared to PSP at 1% (w/v) within 7-days. The higher ES of CSP and PSP than the CFP might be in part due to its greater capacity to increase the emulsion viscosity. However, pectins maintain emulsion stability not only by increasing viscosity around emulsified lipid droplets but also by creating steric hindrance and electrostatic interactions (mainly repulsion) among lipid droplets within the emulsion system through their side and main chains (Funami et al. 2011). The higher steric hindrance created by side chains of CSP and PSP explains its superior ES than CP and AP although the latter had a higher ability to increase viscosity than the former. After purification, emulsification property of fig stalk pectin increased significantly ($p \leq 0.05$). The amounts of protein of CSP, PSP and CP determined in the current work were not considerably different. Thus, it appeared that the differences between ESs of CSP, PSP and CP were also related to their variations in Mw and molecular structure (e.g. HG and RG-I composition, linearity, RG-I branching, repulsion created by negatively charged carboxyl groups, etc.) and/or surface activity of protein constituent.

The particle size characteristics of emulsions, D[3,2], formed at 3% (w/v) pectin concentration are also shown at 0 and 7 days (Fig. 4.2D). On day 0, D[3,2] of pectin emulsions changed between 17 and 80 µm. The smallest sized emulsion droplet at day 0 was observed for CFP (D[3,2]=17 µm) while emulsions of CSP and PSP followed CFP with their smallest sized droplets (D[3,2]=24 µm), and CP emulsion formed the largest sized emulsions (D[3,2]=80 µm). The differences between the mean diameters of fig and citrus pectin emulsions could be related to different factors. For example, the smaller mean diameter of CFP emulsions could be related to its higher protein content (natural surface-active agents) than the others. It is well-known that some polysaccharides such as sugar beet pectin, soluble soybean polysaccharide, and gum Arabic owe their emulsifying properties to the surface activity of complex hydrophobic protein components (Ahmet Yemenicioğlu et al. 2020). In contrast, the large size of CP emulsion droplets might be related to its structural and conformational differences than the fig pectins (Neckebroeck et al. 2021). It appears that the long smooth chains of CP composed of GA units might initially form a thick layer around the oil droplets, thus, resulted with increased droplet sizes (Funami et al. 2011). No significant changes occurred in the mean diameter of CSP emulsion droplets at day 7 while the D[3,2] of CFP emulsions reduced

slightly from 17 to 12 µm. In contrast, the significant reduction observed in the D[3,2] of CP emulsion from 80 to 44 µm suggested the depletion flocculation of pectin molecules from the interface that reduced the thick pectin layer around emulsified lipid droplets and caused the reduction of average droplet size (Neckebroeck et al. 2021) ($p \leq 0.05$). However, the stability of CP emulsion has not been considerably affected by this size change indicating that the sufficient emulsion stabilizing capacity of pectin molecules was still deposited around the oil-water interface of lipid droplets. Besides, the AP and PSP emulsions showed significant increases for 1.6 and 1.4-fold after 7 days, respectively ($p \leq 0.05$) without showing any phase separation. The overall results showed that the fig stalk pectin could be an alternative to commercial citrus pectin to increase the emulsion stability of oil-in-water emulsion foods.

4.4.3. Viscosity

The changes in shear stress versus the shear rate of pectin solutions are shown in Figure 4.3. In general, all pectin solutions showed Newtonian behavior and R^2 values of the curves were calculated as 0.9930, 0.9938, 0.9808, 0.9757, and 0.9956 for AP, CP, CSP, CFP, and PSP respectively. Shear stress versus shear rate linearly increased. During mixing, the pectin chains were attached to each other in the dispersion and entangled, resulting in an increase in viscosity as expected (Lira-Ortiz et al. 2014). Similar results had been observed in studies on commercial citrus pectin and soy pectin (Monsoor 2005). The viscosities of the pectin solutions were also calculated using the slopes of the obtained curves (Figure 4.4) and found in a range from 68 to 10 mPa.s. The AP showed the highest viscosity, while the other viscosities of the samples were determined in decreasing order as PSP, CP, CSP and CFP. These results showed that PSP has better potential than crude fig pectins to be used as thickener than commercial pectins. However, it is noteworthy that CSP had a significantly higher viscosity than CFP but lower to that of CP ($p \leq 0.05$).

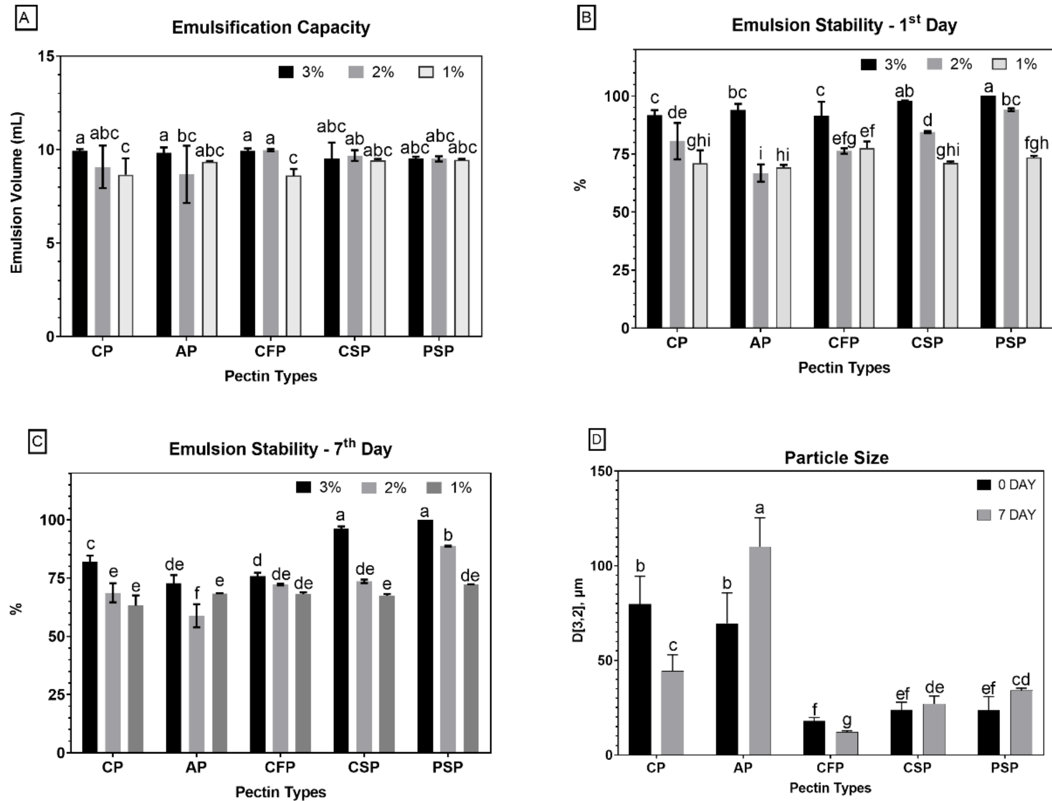

Figure 4.2. Emulsification capacity (A), emulsion stability (1st Day of emulsion stability (B); 7th Day of emulsion stability (C)) and droplet sizes (D) of commercial and different fig pectin emulsions at different concentrations (1-3%, w/v) during room temperature storage at 25 °C. Each data point is shown as the average of three replicates. The error bar indicates standard deviation. Different letters within experiment represent significant difference at $p \leq 0.05$.

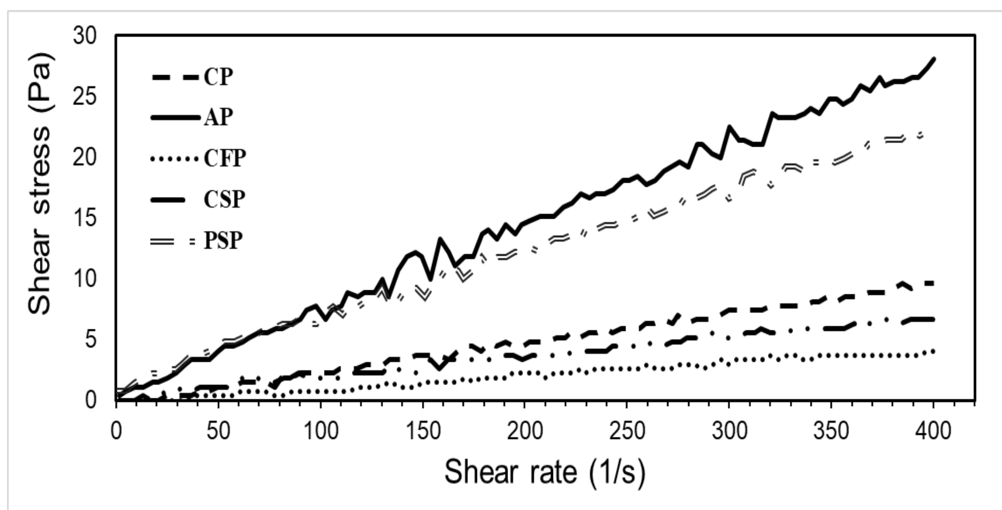

Figure 4.3. Flow behavior of 3% (w/v) solutions of commercial pectins and different fig pectins at 23 °C.

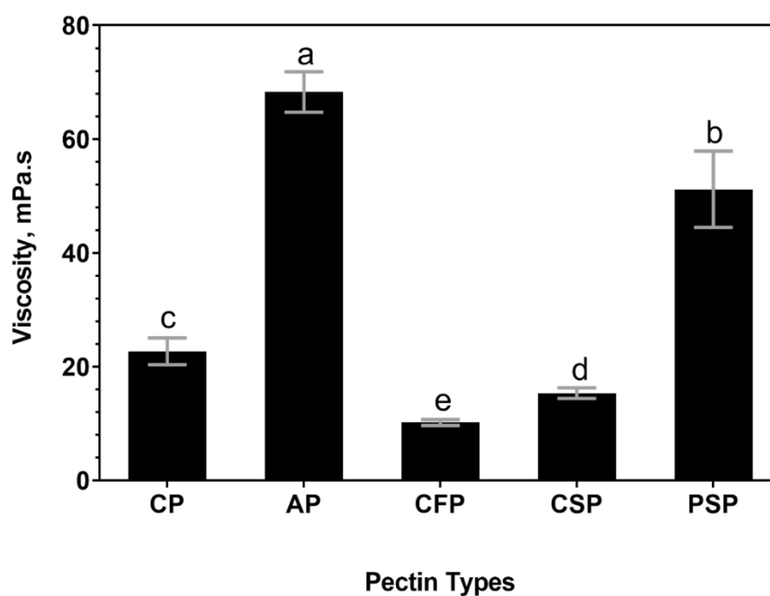

Figure 4.4. Viscosity values of commercial pectins and different fig pectins at 3% (w/v) concentration. Each data point is shown as the average of three replicates. The error bar indicates standard deviation. Different letters represent significant difference at $p \leq 0.05$.

4.4.4. Gelling Capacity

The gel formation capacity of fig pectins was determined by finding the least gelling concentration (LGC). For this purpose, a series of pectin solutions were prepared by high ester pectin jelly preparation (concentrations between 0.4 and 1.2 g/100 g) as described in section 4.3.4. The gel formation was detected by observing the flow characteristics of tube contents when tubes were turned upside down. The LGC corresponds to the lowest pectin concentration (g/100 g) that gives hard gel with no falling or slipping by gravity when tubes are turned upside down. As shown in Fig. 4.5, when the concentration was between 0.4% and 0.6% (w/w), CFP increased the viscosity but did not show a gelling effect. With the increase of the concentration (0.8%-1%, w/w), gelation occurred in the tube. However, this gel was not stable. When the concentration was 1.2% (w/w), a stable gel, which was not very hard, was obtained. Thus, the CFP having LGC of 1.2 g/100 g showed the best gelling performance.

The DE of CFP was 36.7%, but it was unable to show gelation in the presence of 30% sucrose by the standard low methoxyl pectin gelation method (Food Chemicals Codex 1972). As it is well known that for a low esterified pectin (LMP) to form a gel with calcium chloride, a regular complex, referred to as the "egg-box model," must occur between different LMP chains and calcium atoms. In fact, it is claimed that well-gelling LMP pectins with calcium contain a continuous sequence of a certain number of unesterified carboxyl groups with regular intervals (a number ranging between 6 and 20) rather than randomly distributed unesterified carboxyl groups in galacturonic acid chains (Fraeye, Colle, Vandevenne, Duvetter, Van Buggenhout, et al. 2010). Accordingly, it can be thought that in CFP, the unesterified carboxyl groups were not evenly distributed in orderly series and blocks or were divided frequently by side chains and foreign molecules such as proteins, and thus, they cannot form a gel with calcium. As it was stated in section 3.4.5, the RG-I content of CFP was high.

According to the gelling method by the Food Chemical Codex (1972), the high ester pectin was shown gelling capacity at 0.4% (w/w) concentration. The only CP was shown the gelling ability at this concentration that could be measured with texture analyzer. Thus, the LGCs of CP, AP, CFP, CSP and PSP by the standard high methoxyl

pectin gelation method at 64% sucrose were found as 0.4, 2, 1.2, 1.75, and 1.2% (w/w), respectively (Fig. 4.5). Below these concentrations, pectins were only increased the viscosity of the gelling solutions. The pectin concentrations between 1.25 and 3% (w/w) for CFP and PSP with 1.75 and 3% (w/w) for CSP were selected to compare TPAs with those of CP gels from 1.25% to 3% (w/w) and AP gels from 2% to 3% (w/w).

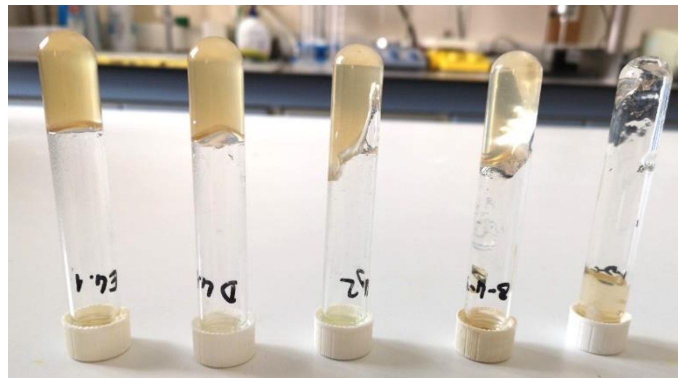

Figure 4.5. The least gelling concentration of CFP gels. The pectin concentration (%, w/w) decreased from left to right as follows: 1.2, 1, 0.8, 0.6 and 0.4%.

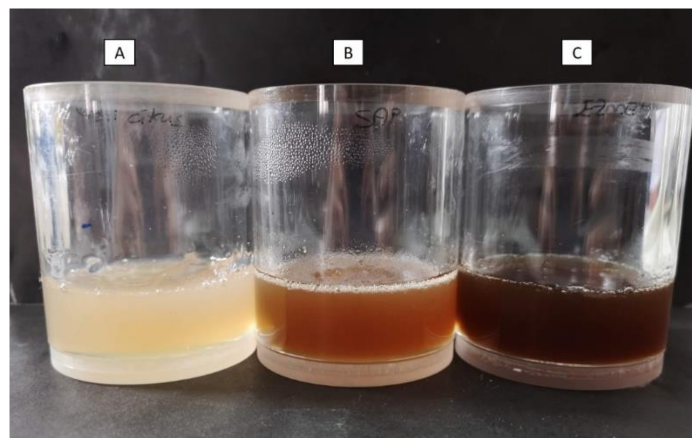

Figure 4.6. Photographic images of CP (A), CSP (B) and CFP (C) gels.

After the LGC concentrations were determined, the gels were prepared according to the standard high ester pectin gel preparation method and poured into the back-extrusion cups in increasing concentrations for each pectin to determine the texture properties of the gels. Photographs of some prepared pectin gels are shown in Figure 4.6. The texture profile analysis (TPA) results of pectin gels are given in Table 4.1. In TPA, the maximum force observed when compression applied to a gel is referred to hardness, while fracturability/brittleness represents a gel's tendency to break, crack, and disperse in response to applied force. A gel that appears hard before force is applied but breaks or cracks instantly when even a small force is applied means that gel is fracturable or brittle. The fracturability could not have been determined for the CP and AP since these pectins did not form brittle gels. In contrast, the CFP, CSP and PSP gave brittle gels with fracturability values ranging between 0.30 and 4.22 N. The 2.4 to 4.7-fold greater fracturability values of the CFP clearly showed that the CFP gels are considerably more brittle than the CSP compared to PSP (0.9 to 1.2-fold higher fracturability). Additionally, the highest gel hardness was also obtained for CFP followed by PSP, CP, CSP and AP. In fact, while a very high hardness was determined at the level of 4.99 N with CFP gel at 3% concentration, the hardness of the gel formed by AP gel at 2% (w/w) concentration was found to be as small as 0.17 N. CP, CSP and PSP formed gels between these two gels. Both the hardness and fracturability of CFP increased at a concentration-dependent manner between 1.25 and 3% (w/w). In contrast, the hardness of CP gels, and the hardness and fracturability of CFP and PSP gels did not change considerably up to 2% (w/w) ($p > 0.05$). On the other hand, AP formed gels with similar hardness showing no significant differences in the concentration ranges studied ($p > 0.05$). However, pectin concentrations at 2.5 and 3% (w/w) caused significant increases in hardness of CP gels, and in both hardness and fracturability of SP and PSP gels ($p \leq 0.05$). These results show that the gel hardness and brittleness properties of CFP are quite different from commercial pectins and forms hard and brittle gel at high concentration. PSP and CSP, on the other hand, form gels that are less brittle than CFP and more similar to CP in terms of hardness.

Table 4.1 also shows the cohesiveness and surface adhesiveness values of the gels. The cohesiveness reflects the potency of the internal forces composing a gel's body. There is a correlation where the cohesiveness is anticipated to be inversely proportional to its susceptibility to fracture under mechanical stress. Thus, the fracturability of a material can be compared to its reduced cohesiveness. (Garrido, Lozano, and Genovese 2015).

Accordingly, it is an expected result that the non-brittle CP and AP gels showed higher cohesiveness values than the brittle CFP, CSP and PSP pectin gels. While AP gel had the highest cohesiveness value (0.96), the lowest cohesiveness value was measured for PSP (0.30). Similarly, the high fracturable CFP and PSP gels showed no significant differences besides 2% and 2.5% (w/w) ($p > 0.05$). The adhesiveness represents the work required to remove the probe from the gel after touching it without pressing it onto the sample surface. However, in the TPA test, adhesiveness cannot be measured accurately, as the probe is withdrawn from the sample after compression. However, the surface adhesion value determined in the TPA test is still accepted as a measure of the elastic-plastic structure of the sample. When adhesiveness values are examined, it is seen that AP gels do not have a remarkable surface adhesiveness, whereas CP gel has the highest surface adhesiveness. Although CFP gels have less surface adhesiveness than CSP gels, the surface adhesiveness value of PSP gel at 3% (w/w) is not found statistically different from CP gel at 2% (w/w) concentration ($p > 0.05$).

The gumminess and chewiness of CFP gels increased at a concentration-dependent manner between 1.25 and 3% (w/w) while CP, CSP and PSP concentrations must have been increased above 2% (w/w) to have a concentration-dependent increase in these two parameters. The AP showed no significant differences between the concentration for these two parameters ($p > 0.05$) that gave the least gummy gels. Among fig pectins, the SP gave the least gummy with lower chewiness values gels than CFP and PSP. The CFP and PSP gels showed 2.6-4.6- and 0.3-1.6-fold higher gumminess than CSP, respectively.

The CFP, PSP and CP gels had similar gumminess at 1.25 and 1.5% (w/w) but similarity between the CP and CFP continued for 2.5 and 3% (w/w) concentration that PSP gave significantly less gummier gels than these gels from 0.7-fold to 2-fold ($p \leq 0.05$). The chewiness values of different pectins showed high parallelism with gumminess values. Therefore, FP gave the highest chewiness values followed by CP, PSP, CSP and AP.

The springiness values represent the rate at which the gel returns to its original state after the deforming force is removed (Garrido, Lozano, and Genovese 2015). When these values are examined, it is seen that pectin type and concentration have only a limited effect on springiness values. The decreases of springiness with concentration were

associated to decrease the elasticity of the jellies. The resilience values of the gels reflect their ability to return to their initial height with resistance. It was determined that the resilience values of the gels decreased as the pectin concentration increased. AP showed a significantly higher strength than all other gels ($p \leq 0.05$), whereas the strength values of other gels (CP, CFP, CSP, PSP) showed statistically indifferent values from each other below 2% (w/w) concentration ($p > 0.05$).

The overall results of TPA clearly showed that the gels obtained from fig pectins show an exceptional gel characteristic by being much harder, having higher fragility, and forming gummy gels that are different from commercial pectins. It is evident that these unique gel properties carry significance in terms of producing food products with alternative textural properties. The display of different gel characteristics by pectins indicates that their molecular structures and organizations are different. Indeed, it is well known that differences in chain length, methylation degree, neutral sugars linked to side chains, acetylation and among other properties, also affect the textural properties of gels (Thakur, Singh, and Handa 1997). It was reported that as the RG-I side chains increased, the formation of the tighter gelling network was promoted due to the increased entanglements among pectin molecules, and the formation of subsequent hydrophobic interactions and hydrogen bonding among HG chains (Sousa et al. 2015).

4.4.5. Water and Oil Absorption Capacity

The WAC and OAC values of fig pectins and commercial pectins are shown in Table 4.2. The PSP showed the greatest WAC (10 g/g) followed by CSP, CFP, AP and CP. However, the WAC of CFP and AP were not found significantly different ($p > 0.05$). The PSP and CSP also showed higher WAC than those of eggplant peel (6 g/g) and calyx (4.6 g/g) pectins (Kazemi, Khodaiyan, and Hosseini 2019), fiber pectin from tomato pomace (3.57 g/g) (Namir, Siliha, and Ramadan 2015) and olive mill wastewater pectins (3.00 and 2.18 g/g) (Rubio-Senent et al. 2015). Thus, the outstanding WAC of PSP could be employed for moisture binding in food formulations. In contrast, all pectins used in

this study showed low OACs and were not found significantly different ($p > 0.05$). However, the OAC's of fig pectins were comparable to those of pectins extracted from *Opuntia ficus indica* (1.23 g/g) (Bayar, Friji, and Kammoun 2018), eggplant calyx (1.46 g/g) (Kazemi, Khodaiyan, and Hosseini 2019) and walnut green husk (1.21 g/g) (Asgari et al. 2020) while pectin from eggplant peel (2.6 g/g) (Kazemi, Khodaiyan, and Hosseini 2019) and sunflower by-product (2.51 g/g) (Ezzati et al. 2020) showed higher OAC than pectins in the current study.

Table 4.1. Functional properties of commercial pectins and different fig pectins.

Characteristics	**CP**	**AP**	**CFP**	**CSP**	**PSP**
TEAC (µmol Trolox/100g)	64.9 ± 5.25^{c}	26.7 ± 4.91^{d}	79.3 ± 17.2^{bc}	107 ± 31.5^{ab}	143 ± 32.7^{a}
TPC (g GAE/100 g)	0.68 ± 0.07^{c}	0.26 ± 0.03^{d}	1.25 ± 0.08^{b}	1.09 ± 0.05^{b}	1.82 ± 0.42^{a}
WAC (g/g)	3.12 ± 0.12^{d}	3.98 ± 0.78^{cd}	4.15 ± 0.24^{c}	8.42 ± 0.76^{b}	10.0 ± 0.16^{a}
OAC (g/g)	1.27 ± 0.10^{a}	1.27 ± 0.18^{a}	1.67 ± 0.09^{a}	1.48 ± 0.14^{a}	1.43 ± 0.22^{a}

*Data are shown as mean ± standard deviation of triplicate measurement. Data at each column indicated by different letters are significantly different ($p \leq 0.05$). TEAC: Trolox equivalent antioxidant capacity, TPC: total phenolic content, WAC: water absorption capacity, OAC: oil absorption capacity.

Table 4.2. Texture profile analysis of pectin gels at different concentrations.

	Conc (%,w/w)	Fracturability (N)	Hardness (N)	Cohesiveness *	Adhesiveness (N.S)
CP	**1.25**	-	0.45 ± 0.02^{ij}	0.82 ± 0.03^{c}	-0.61 ± 0.07^{cdef}
	1.50	-	0.44 ± 0.04^{ij}	0.77 ± 0.04^{d}	-1.00 ± 0.34^{fgh}
	1.75	-	0.48 ± 0.03^{i}	0.80 ± 0.01^{cd}	-1.39 ± 0.41^{hi}
	2	-	0.55 ± 0.14^{i}	0.80 ± 0.04^{cd}	-1.74 ± 0.49^{ij}
	2.50	-	1.74 ± 0.68^{e}	0.68 ± 0.08^{e}	-4.84 ± 0.10^{n}
	3	-	2.50 ± 0.14^{c}	0.68 ± 0.01^{e}	-7.54 ± 0.42^{o}
AP	**2**	-	0.17 ± 0.00^{l}	0.95 ± 0.01^{ab}	0.12 ± 0.03^{a}
	2.50	-	0.17 ± 0.01^{l}	0.96 ± 0.01^{a}	0.14 ± 0.02^{a}
	3	-	0.19 ± 0.00^{l}	0.92 ± 0.00^{b}	0.04 ± 0.01^{a}
CFP	**1.25**	0.50 ± 0.01^{i}	0.82 ± 0.02^{gh}	0.44 ± 0.01^{hi}	-0.21 ± 0.02^{abc}
	1.50	0.77 ± 0.02^{h}	1.27 ± 0.10^{f}	0.42 ± 0.02^{ij}	-0.40 ± 0.00^{bcd}
	1.75	1.12 ± 0.03^{g}	1.86 ± 0.00^{de}	0.41 ± 0.01^{ijk}	-0.67 ± 0.02^{def}
	2	1.66 ± 0.01^{e}	2.30 ± 0.27^{cd}	0.39 ± 0.01^{jkl}	-1.15 ± 0.02^{gh}
	2.50	2.63 ± 0.09^{d}	3.48 ± 0.58^{b}	0.36 ± 0.04^{lmn}	-2.17 ± 0.00^{jk}
	3	4.22 ± 0.09^{a}	4.99 ± 0.58^{a}	0.37 ± 0.03^{lmn}	-4.16 ± 0.72^{lm}
CSP	**1.75**	0.30 ± 0.03^{k}	0.31 ± 0.06^{k}	0.57 ± 0.04^{f}	-0.10 ± 0.06^{ab}
	2	0.36 ± 0.07^{j}	0.38 ± 0.10^{jk}	0.52 ± 0.06^{g}	-0.23 ± 0.26^{abc}
	2.50	1.05 ± 0.05^{g}	1.26 ± 0.00^{f}	0.36 ± 0.02^{lmn}	-2.18 ± 0.17^{k}
	3	1.79 ± 0.33^{e}	1.99 ± 0.36^{de}	0.36 ± 0.01^{lmn}	-4.11 ± 0.54^{lm}
PSP	**1.25**	0.54 ± 0.02^{i}	0.68 ± 0.02^{h}	0.47 ± 0.00^{h}	-0.57 ± 0.02^{cde}
	1.50	0.56 ± 0.02^{i}	0.77 ± 0.07^{h}	0.42 ± 0.02^{i}	-0.89 ± 0.24^{efg}
	1.75	0.87 ± 0.07^{h}	0.10 ± 0.17^{g}	0.38 ± 0.02^{klm}	-1.28 ± 0.33^{gh}
	2	1.40 ± 0.39^{f}	2.00 ± 0.69^{de}	0.34 ± 0.04^{n}	-3.78 ± 0.34^{l}
	2.50	3.02 ± 0.10^{c}	3.27 ± 0.11^{b}	0.30 ± 0.00^{o}	-4.24 ± 0.07^{m}
	3	3.44 ± 0.23^{b}	3.52 ± 1.87^{b}	0.35 ± 0.01^{mn}	-4.76 ± 0.07^{n}

(cont. on next page)

Table 4.2. (cont.).

	Conc (%,w/w)	Gumminess (N)	Springiness (Mm)	Chewiness (N.Mm)	Resilience *
CP	**1.25**	0.37 ± 0.03^{hijk}	0.98 ± 0.01^{bcde}	0.36 ± 0.04^{hi}	0.18 ± 0.01^{d}
	1.50	0.34 ± 0.02^{hijklm}	0.97 ± 0.04^{efg}	0.33 ± 0.03^{i}	0.18 ± 0.03^{de}
	1.75	0.38 ± 0.02^{hij}	0.95 ± 0.01^{ghi}	0.36 ± 0.02^{hi}	0.13 ± 0.00^{f}
	2	0.44 ± 0.09^{hi}	0.94 ± 0.00^{ijk}	0.41 ± 0.09^{h}	0.11 ± 0.04^{fghi}
	2.50	1.15 ± 0.32^{bc}	0.92 ± 0.00^{k}	1.05 ± 0.30^{bcd}	0.13 ± 0.02^{fg}
	3	1.69 ± 0.12^{a}	0.94 ± 0.00^{ijk}	1.58 ± 0.12^{a}	0.12 ± 0.01^{fg}
AP	**2**	0.16 ± 0.01^{m}	1.00 ± 0.00^{abcd}	0.16 ± 0.01^{k}	0.65 ± 0.08^{a}
	2.50	0.16 ± 0.01^{m}	1.00 ± 0.00^{a}	0.16 ± 0.00^{jk}	0.69 ± 0.06^{a}
	3	0.18 ± 0.00^{lm}	1.01 ± 0.00^{a}	0.18 ± 0.00^{jk}	0.41 ± 0.01^{b}
CFP	**1.25**	0.36 ± 0.00^{hijkl}	0.98 ± 0.01^{cde}	0.35 ± 0.01^{hi}	0.11 ± 0.00^{fgh}
	1.50	0.53 ± 0.06^{gh}	0.97 ± 0.00^{ef}	0.52 ± 0.06^{g}	0.11 ± 0.01^{fgh}
	1.75	0.76 ± 0.02^{ef}	0.97 ± 0.01^{efgh}	0.74 ± 0.01^{ef}	0.10 ± 0.01^{ghi}
	2	0.89 ± 0.12^{de}	0.96 ± 0.00^{efgh}	0.86 ± 0.12^{de}	0.09 ± 0.01^{hij}
	2.50	1.27 ± 0.35^{b}	0.96 ± 0.01^{fgh}	1.21 ± 0.32^{b}	0.08 ± 0.02^{jk}
	3	1.82 ± 0.06^{a}	0.97 ± 0.00^{efg}	1.76 ± 0.07^{a}	0.08 ± 0.02^{ij}
CSP	**1.75**	0.17 ± 0.02^{lm}	1.00 ± 0.01^{abc}	0.17 ± 0.02^{jk}	0.18 ± 0.05^{d}
	2	0.20 ± 0.03^{jklm}	1.00 ± 0.01^{ab}	0.20 ± 0.03^{j}	0.14 ± 0.05^{ef}
	2.50	0.45 ± 0.03^{hi}	0.95 ± 0.00^{hij}	0.42 ± 0.03^{h}	0.06 ± 0.00^{kl}
	3	0.71 ± 0.10^{efg}	0.94 ± 0.01^{ijk}	0.66 ± 0.09^{f}	0.05 ± 0.00^{l}
PSP	**1.25**	0.32 ± 0.01^{ijklm}	0.98 ± 0.00^{de}	0.31 ± 0.01^{i}	0.09 ± 0.00^{hij}
	1.50	0.32 ± 0.02^{ijklm}	0.96 ± 0.01^{fgh}	0.31 ± 0.01^{i}	0.08 ± 0.01^{jk}
	1.75	0.38 ± 0.08hij	0.98 ± 0.03^{ef}	0.37 ± 0.07^{hi}	0.07 ± 0.01^{jk}
	2	0.67 ± 0.16^{fg}	0.97 ± 0.05^{ef}	0.65 ± 0.18^{f}	0.13 ± 0.09^{fg}
	2.50	1.00 ± 0.04^{cd}	0.97 ± 0.04^{efg}	0.97 ± 0.08^{cd}	0.32 ± 0.01^{b}
	3	1.23 ± 0.63^{b}	0.93 ± 0.00^{jk}	1.15 ± 0.58^{bc}	0.24 ± 0.07^{c}

*Data are shown as mean ± standard deviation of triplicate measurement. Data at each column indicated by different letters are significantly different ($p \leq 0.05$).

4.4.6. Antioxidant Activity

The TEAC based free radical scavenging activity of fig and commercial pectins originated from their polysaccharide structure (e.g, -OH and -COOH groups) (Gharibzahedi et al., 2019b; Wang et al., 2016) and bound antioxidant components (e.g., Maillard reaction products, proteins and polyphenols) (Domínguez Avila et al. 2018) were given in Table 4.2. The PSP showed almost 5.4 to 1.3-fold higher antioxidant activity than other pectins. However, no significant differences existed between the antioxidant activities of PSP and CSP ($p > 0.05$). While the TEAC results of CP and CFP were found similar, the result of AP was found the lowest of all. Similarly, as shown in Table 4.2, the TPC content of PSP was found to be greater than other pectins. The TPC of CFP and CSP was found to be similar and higher than commercial pectins. Due to differences in antioxidant activity determination methods and expression of results, it is difficult to compare the antioxidant activity of fig pectins with other pectins. However, pectin from fresh fig peel was also reported to show free radical scavenging based on antioxidant capacity (Gharibzahedi et al. 2019b). Some other pectins with reported free radical scavenging activity include okra pectin (Xu et al. 2020), mangosteen rind pectin (Wathoni et al. 2019), grapefruit pectin (Wang et al. 2016) and sweet potato pectin (Ogutu and Mu 2017).

The sun-dried Sarılop figs used in the current study are also known as a good source of polyphenols (Kelebek et al. 2018). The polyphenols form a complex with polysaccharides like pectin through hydrophobic interactions (Liu, Le Bourvellec, and Renard 2020; Tang, Covington, and Hancock 2003). After that, the complex is stabilized with hydrogen bonds formed mainly between hydroxyl groups of polyphenols and oxygen atoms in different groups/linkages of polysaccharides (e.g., carboxyl/carboxylic acid and hydroxyl groups, the oxygen atom of glycosidic linkages) (Jakobek 2015; Liu, Le Bourvellec, and Renard 2020; Wu et al. 2009). In the current study, the highest total phenolic content (TPC) was determined for PSP (1.82 g GAE/100 g), followed in descending order by CFP, CSP, CP, and AP. However, it should be noted that the TPCs of fig pectins in the current work were significantly lower than the TPC of ~3.0 g

GAE/100 g determined for pectin extracted from peels of fresh figs (Gharibzahedi, Smith, and Guo 2019b).

4.5. Conclusions

This work revealed that the stalks separated as waste during the processing of sun-dried figs are a better source of pectin than severely defected low-grade (substandard) dried figs since they provided higher extraction yield, degree of esterification, galacturonic acid content, and homogeneity (higher HG, but lower RG and branching). The characterization of stalk waste pectin and comparison of its functional properties with those of commercial citrus pectin clearly showed that this hydrocolloid presents outstanding emulsion stability, water absorption capacity, and alternative gelling properties. The fig stalk waste pectin also possesses almost 2-fold higher free radical scavenging-based antioxidant activity than citrus pectin. This work revealed for the first time that there is a good potential of producing value-added pectin products from sun-dried fig processing wastes. The fig stalk waste pectin could be utilized for developing functional foods such as yogurts, yogurt and fruit beverages, dressings and sauces, smoothie balls, jams, and jellies having alternative structural and rheological properties.

CHAPTER 5

DEVELOPMENT AND CHARACTERIZATION OF EDIBLE FILM FROM FIG PECTIN

5.1. Introduction

The edible films from pectin have been attracting increasing interest since this hydrocolloid might be used to produce different types of edible packaging such as solution-cast or compression molded self-standing films (Oliveira et al. 2021), extruded casings (Liu, Kerry, and Kerry 2007) or edible coatings (Çavdaroğlu, Farris, and Yemenicioğlu 2020). Commercial citrus and apple pectins as well as pectin from alternative sources (e.g., coffee, mango peel, passion fruit peel, hawthorn pectins, pineapple peel, lime peel, red pomelo peel) have been recently used in the development of edible packaging materials (Chamyuang et al. 2021; Henao-Díaz et al. 2021; Lozano-Grande et al. 2016; Nisar et al. 2018; Ribeiro et al. 2021; Rodsamran and Sothornvit 2019a; 2019b; Sood and Saini 2022). Moreover, different studies have also been performed to improve the poor mechanical and barrier (water vapor and oxygen permeability) properties of pectin films by using composite film making strategies employing alternative hydrocolloids (e.g., pumpkin protein extract, chitosan, hydroxypropyl methylcellulose, carboxymethylcellulose) (Dranca et al. 2021; Lalnunthari, Devi, and Badwaik 2020; Lozano-Grande et al. 2016; Rincón et al. 2021) or waxes (Lozano-Grande et al. 2016). Cross-linking is also an alternative strategy that could

be used to improve the mechanical and barrier properties of pectin films. Pectin is an anionic polysaccharide that can be cross-linked with divalent cations such as calcium (Ca^{++}) and magnesium (Mg^{++}) (Li and Buschle-Diller 2017; Moslemi 2021). The cross-linking formed as a result of extensive junction zones among divalent ions and de-esterified carboxyl groups of pectin increases the mechanical strength of resulting hydrogels while decreasing their water solubility (Rezvanain et al. 2017). The cross-linking occurs extensively in low methoxyl pectins (degree of esterification < 50%) and the formed network is generally explained by the classical "egg-box" model (D. E. Ngouémazong et al. 2012).

In this chapter the characteristics of novel fig pectin edible films were explored. For this purpose, pristine and $CaCl_2$ cross-linked films of crude pectin from whole low-grade sun-dried figs and crude and purified pectins from stalk wastes separated during processing of high-quality sun-dried figs were evaluated for their detailed physicochemical properties such as solubility, swelling, hydrophobicity, mechanical and barrier properties, color and transparency, and morphological features. The properties of fig pectin films were also compared with those of commercial citrus and apple pectin films. The relevance of this work lies in the fact that it is the first study showing the advantages of fig pectin edible films over currently used commercial pectin films. Moreover, this is the first report that investigates the effect of pectin composition and molecular properties on the physicochemical characteristics of obtained edible films by analyzing Pearson's correlation coefficients. Besides, the results presented in this chapter were already published by Çavdaroğlu et al. (2023).

5.2. Materials

Citrus pectin (P9135, 79% GA) was obtained from Sigma-Aldrich (St. Louis, MO, USA). Apple pectin was obtained from Tito (İzmir, Turkey). All other chemicals were reagent grade. The cut stalk waste (contains stalk and a piece of fruit flesh that accounts

for 1 to 1.5% of total fruit flesh weight) of high-quality sun-dried figs (Cultivar Sarılop, UNECE class I, size number 9 and 10) separated during processing (fig stalk), and the low quality (UNECE substandard) sun-dried figs (Cultivar Sarılop) which are mainly processed into pastes were kindly supplied by KFC Gıda A.Ş (Menemen, Turkey). All samples used were fluorescence tested in the factory to ensure that they were free from mycotoxins. The samples were kept at -20 ºC until they were used for pectin extraction.

5.3. Methods

The methods given below were performed to characterize the edible film forming properties of fig pectins and compared with those of commercial pectins.

5.3.1. Film Forming

For the preparation of films, solutions of different pectins at 3% (w/v) were heated using a hot plate working under continuous stirring at 60 °C for 30 min. The solutions were then cooled to room temperature and further treated at 10,000 rpm for 1 min using a homogenizer-disperser (Heidolph, Germany, rotor ϕ = 6.6 mm Tip). Then, 0.9 g glycerol (30% of pectin, w/w) was added as a plasticizer, and the mixture was stirred for 15 min. The solution was further homogenized at 10,000 rpm for 4 min using the homogenizer-disperser. To obtain solution-cast pristine films, 20 g portions of film solutions were poured into glass Petri dishes (inner diameter 10 cm) and the dishes were dried in a controlled test cabinet at 25 °C and 50% RH for 24 h. The cross-linked films were obtained by treating dried films with 3% (w/w) $CaCl_2$ solution and drying films

again in the controlled test cabinet at 25 °C and 50% RH for 24 h (Rezvanain et al. 2017). The pristine and cross-linked films of crude pectins from low-grade (substandard) dried fig and stalk waste were designated CFP and CFP-Ca^{++}, and CSP and CSP-Ca^{++}, while pristine and cross-linked films of purified pectin from stalk were designated PSP and PSP-Ca^{++}, respectively. The pristine and cross-linked commercial citrus and apple pectins films were designated CP and CP-Ca^{++}, and AP and AP-Ca^{++}, respectively. All pectin films were prepared in duplicate.

5.3.2. Mechanical Tests

Tensile strength at break (TS), elongation at break (EAB), and Young's modulus (YM) were determined using Texture Analyzer TA-XT2 (Stable Microsystems, Godalming, UK) according to ASTM Standard Method D882-02 (2002a). The dried films were conditioned in a controlled test cabinet at 25 °C, 50% RH for 24 h before testing. Then, the films were cut into 50-mm-long and 8-mm-wide strips. The initial grip distance was 50 mm, and the drawing speed was 50 mm/min. An average of eight measurements was taken. At least eight strips of each film were tested, and films were prepared with duplicated. The thickness of films was measured by using a micrometer (Chronos, UK).

5.3.3. Determination of Water Vapor Permeability

The water vapor permeability (WVP) of pectin films was measured using Payne permeability cups (Elcometer 5100, England) according to the ASTM Standard Method E96 (2016). Each cup was filled with 3 g of dried silica beads. The thickness of the

samples was measured. Each film sample with a diameter of about 6 cm was cut and placed on top of the cups and sealed with three tight clamps after putting the O-ring. These cups were weighed then placed in a controlled test cabinet (TK 120, Nüve, Turkey) at 25 °C and 60% RH and the start time were recorded. The cups were weighted periodically for 72 h. Cups were weighed at scheduled times. The weight increase of the cups was plotted against time and the linear portion of the curve using the last five data points resulted in $R^2 \geq 0.99$ was taken for calculation of WVP according to the following equation:

$$WVP = \frac{G\ L}{A\ t\ S\ (RH_1 - RH_2)}$$

where G is the weight change from the straight line (g), L is the thickness of the film (mm), t is the time (day), A is the test area (m^2), S is the saturation vapor pressure at test temperature (3.169 kPa at 25 °C), RH_1 the relative humidity of the test chamber (60%) and RH_2 the relative humidity in the dish (0%). Four independent tests per film were performed.

5.3.4. Moisture Content and Solubility in Water

Before determining their solubilities, the moisture content of the films was determined by the vacuum oven method applied at 70 ºC and 16.9 kPa for 24 h. Eight pieces of each film were measured for their moisture contents. The solubility of films was determined according to the method described by Pérez et al. (2016). Briefly, pieces of films (15 × 7.5 mm^2) were placed into a test tube with 10 mL of distilled water. The tubes were then shaken at 240 rpm for 24 h using an orbital shaker (IKA, OS 5 basic, Germany) and placed in an incubator at 25 °C and 50% RH. After that, the remaining solids in the tubes were collected by filtration. The insoluble dry matter content was determined by hot air drying at 105 °C until reaching constant weight. Eight pieces from each film were

tested for their solubility. The film solubility (%) was determined according to following equation:

$$\text{Film solubility (\%)} = 100 \times \frac{\text{(Initial dry matter-Insoluble dry matter)}}{\text{Initial dry matter}}$$

5.3.5. Swelling Degree

The swelling degree of films was determined based on the gravimetric method after the films were held in distilled water for 7.5, 15 and 30 min. After each time interval, the weights of films were determined at room temperature. Measurements were made in three repeats for two replicates. The percentage of swelling degree (SW) was determined using the following equation:

$$SW = 100\left(\frac{(W_W - W_D)}{W_D}\right)$$

where W_D is the weight of dried film; W_W is the weight of the swelling film.

5.3.6. Transparency and Color of Films

Film transparency was determined according to ASTM D-1746 (2002b) with modifications of the method described by Pérez et al. (2016). The transparency of dried films was measured at 600 nm using a spectrophotometer. Rectangular pieces of films

(10 x 30 mm) were placed on the internal side of a spectrophotometer cell and the empty cell was used as a control. Eight replicates of each film formulation were tested. Transparency (T_{600}) was calculated as the following equation:

$$T_{600}=\frac{(\log T\%)}{b}$$

where T is the transmittance and b is the film thickness (mm).

Color measurements were determined using the Minolta CR-400 colorimeter (Minolta Sensing, Osaka, Japan) by determining the L*, a*, b* values. Measurements were carried out in triplicate and averages are reported.

5.3.7. Morphology of Film

The surface and cross-sectional morphologies of pectin films were examined by using a scanning electron microscope (SEM, 250 Quanta FEG, FEI Company, USA). Before the experiment, the films were freeze-dried and then placed into liquid nitrogen and crashed for the SEM examination. Specimens were gold-coated with a sputter coater (Emitech K550X, Quorum Technologies Inc., UK) under 10 mA for 60 s.

The surface images of control and crosslinked films were carried out by an atomic force microscope (AFM) (MMCSPM Nanoscope 8 from Bruker, USA) in an intermittent-contact mode in the air with silicon tips (resonance frequency ≈340 kHz, spring constant ≈40 N/m, tip radius 8 nm). The captured images (min 4 for each sample) were analyzed by Nanoscope Analysis software v.1.5 (Bruker, USA). The surface roughness R_{rms} was calculated as the root mean square average of height deviations (Zi) taken from a mean data plane (Z).

$$R_{rms}=\sqrt{\frac{\sum_{i=1}^{N}\left(Z_i-\underline{Z}\right)^2}{N-1}}$$

The R_{max} parameter indicates the maximum vertical distance between the highest and the lowest points in the image.

5.3.8. Statistical Analysis

Statistical difference between treatments was determined by using variance analysis (one way-ANOVA) and Fisher post-test ($p \leq 0.05$) using Minitab (ver.18.1, Minitab Inc., United Kingdom). Pearson's correlation tests were carried out to investigate the interrelationships of the pectin's compositional profile and their film's mechanical properties.

5.4. Result and Discussion

The detailed results and discussion will be given in the following sections.

5.4.1. Mechanical Properties of Pectin Films

Mechanical properties of pristine and $CaCl_2$ cross-linked films obtained from fig and commercial pectins are shown in Table 5.1. Although the cross-linking caused a significant reduction in thickness of all films, the average thicknesses of pristine and cross-linked films of citrus and fig pectins changed at a very narrow range between 84.1 and 89.0 µm and 71.2 and 78.4 µm, respectively. In contrast, pristine and cross-linked AP films were significantly thinner than all of the other respective pectin films. The results obtained for pristine films showed that the CP and PSP films had the highest tensile strengths (TSs), while AP and CSP films showed 2.4-2.8-fold lower TSs, and CFP showed 5-5.7-fold lower TS than those of CP and PSP films. The cross-linking improved the TSs of some films significantly. For example, AP-Ca^{++}, CP-Ca^{++}, and CFP-Ca^{++} films showed 1.3-, 1.6- and 1.7-fold higher TSs than their pristine AP, CP, and CFP films, respectively. In contrast, no significant differences were determined between TSs of CSP and CSP-Ca^{++} and PSP and PSP-Ca^{++}. It is hard to understand the exact reason for failed $CaCl_2$ cross-linking to improve TS of stalk pectin films. However, it seemed that the crude and purified stalk waste pectins lacked a block-wise distribution for deesterified carboxyl groups that were essential for the formation of a highly ordered mechanically stable egg-box structure (Fraeye et al. 2009). The CP-Ca^{++} showed the highest TS among cross-linked films followed in descending order by TSs of PSP-Ca^{++}, AP-Ca^{++}, CSP-Ca^{++}, and CFP-Ca^{++}. The CSP, AP, and CFP gave the most flexible pristine films with elongation at break (EAB) values of 26.2, 21.9, and 15.2%, respectively. The PSP films showed limited flexibility (EAB: 8.8%), while CP gave almost no flexibility (EAB: 4.2%). The cross-linking caused a significant reduction (1.8 to 3.7-fold) in EAB of most pectin films, except for CP films that gave similar EAB for pristine and cross-linked films. According to Young's modulus (YM) values, the CP-Ca^{++} and PSP-Ca^{++} films were the stiffest films, followed in descending order by CP and PSP films showing intermediate stiffness and by AP-Ca^{++}, CSP-Ca^{++}, CP-Ca^{++}, CSP, CFP, AP films showing lower-intermediate to low stiffness. The overall results clearly showed that pristine and cross-linked films of PSP and CP showed similar mechanical properties, whereas pristine and cross-linked films of CSP and CFP showed similar or slightly different mechanical properties with AP.

Moreover, it is also evident that the purification of CSP and use of obtained PSP in film making caused significant improvements in the mechanical strength of fig stalk waste pectin films.

In the literature, edible films from different pectins have also been characterized for their mechanical properties. For example, pristine films from pomegranate peel (3%, w/v), pineapple peel (3%, w/v), and lime peel (1%, w/v) pectins showed TS values of 2.42, 5.60, and 16.93 MPa, and EAB values of 6.55, 14.84 and 1.77%, respectively (Oliveira et al. 2016; Rodsamran and Sothornvit 2019a; 2019b). These results suggested that the pristine films of pomegranate peel and pineapple peel pectins had comparable mechanical properties with pristine films of CFP and CSP pectins, respectively, while films of lime peel pectin showed comparable mechanical properties with films of PSP. Data on mechanical properties related to $CaCl_2$ cross-linked films of alternative novel pectins derived from wastes are limited. It was reported that the pectin obtained from mango peel could not form a film in the presence of $CaCl_2$ (Chaiwarit et al. 2020). In contrast, edible films obtained from pumpkin peel pectin (5%, w/v) showed a TS of 5.28 MPa and EAB of 14.37% after $CaCl_2$ cross-linking (Lalnunthari, Devi, and Badwaik 2020). Furthermore, in a study, cross-linking with 1% $CaCl_2$ increased the tensile strength of the commercial citrus pectin films by 1.6-fold while decreasing the elongation at break and water solubility by 1.4 and 1.6-fold, respectively (Hari et al. 2021). Similarly, a 70% increase in the toughness and 50% increase in the tensile strength were shown after PVA/commercial citrus pectin blended films were cross-linked with 4.0 wt% $CaCl_2$ solution (John, Deshpande, ad Varughese 2021).

The calculated Pearson's coefficient of correlations (r) showed the factors (composition and molecular properties of pectins) affecting the mechanical properties of pristine and cross-linked films separately shown in Table 5.2 (also see Appendix B as Table B.1 and B.2). The most significant positive correlations were determined between GA of pectins and TS (r = 0.802 and 0.847) and YMs (r = 0.717 and 0.852) of pristine and cross-linked films, respectively. The calculated HG content of pectins also showed moderately significant positive correlations with TS (r = 0.760) and YM (r = 0.790) of cross-linked films, but HG content of pectins showed less significant ($r < 0.7$) correlations with TS and YM of pristine films. Moreover, a moderate positive correlation was also determined between the DE of pectins and TS of pristine films (r = 0.726). As expected, moderately significant negative correlations also existed between GA of pectins and EAB

of pristine (r = -0.734) and cross-linked (r = -0.794) films. These results clearly showed that the GA is the primary factor giving the mechanical strength and stiffness of both pristine and cross-linked films. The R-2 of pectins also showed a moderately significant positive correlation with EAB of pristine and cross-linked films (r = 0.728 and 0.727), respectively. This correlation is expected since R-2 was inversely proportional to the GA content of pectins. In contrast, the calculated RG-I content of pristine films did not correlate with EAB, while a weak positive correlation existed between RG-I content and EAB or cross-linked films (r = 0.595).

Table 5.1. Mechanical properties of commercial pectin and different fig pectin films.

Film sample	**Thickness (μm)**	**Tensile Strength (MPa)**	**Elongation at break (%)**	**Young's modulus (MPa)**
CP	87.8 ± 2.04^{a}	17.6 ± 2.50^{b}	4.18 ± 1.74^{f}	7.56 ± 0.87^{bc}
CP-Ca^{++}	74.7 ± 3.20^{cd}	27.7 ± 2.56^{a}	3.51 ± 0.53^{f}	11.9 ± 1.42^{a}
AP	71.3 ± 0.13^{d}	6.29 ± 0.31^{e}	21.9 ± 7.70^{ab}	0.52 ± 0.12^{f}
AP-Ca^{++}	59.6 ± 7.41^{e}	8.40 ± 2.77^{c}	5.87 ± 1.00^{e}	2.77 ± 0.47^{d}
CFP	89.0 ± 11.7^{a}	3.11 ± 0.36^{f}	15.2 ± 1.23^{bc}	0.55 ± 0.05^{f}
CFP-Ca^{++}	71.2 ± 1.36^{d}	5.34 ± 0.06^{e}	7.88 ± 1.61^{de}	1.54 ± 0.27^{e}
CSP	84.1 ± 1.94^{ab}	6.50 ± 1.71^{de}	26.2 ± 0.77^{a}	0.70 ± 0.12^{f}
CSP-Ca^{++}	71.9 ± 5.81^{d}	7.84 ± 1.23^{cd}	14.3 ± 5.51^{c}	1.55 ± 0.33^{e}
PSP	85.8 ± 3.61^{a}	15.6 ± 1.02^{b}	8.83 ± 1.37^{d}	5.69 ± 1.18^{c}
PSP-Ca^{++}	78.4 ± 1.56^{bc}	19.1 ± 2.52^{b}	4.19 ± 1.34^{f}	8.72 ± 0.52^{ab}

*Data are shown as mean ± standard deviation of triplicate measurement. Data at each column indicated by different letters are significantly different ($p \leq 0.05$). -Ca++ denoted for crosslinks for all pectins.

Table 5.2. Correlations between mechanical and molecular properties of commercial pectin and different fig pectin films.

Property	Tensile Strength (MPa)		Elongation at break (%)		Young's modulus (MPa)	
	Pristine	*Crosslinked*	*Pristine*	*Crosslinked*	*Pristine*	*Crosslinked*
GA	0.802, 0.000	0.847, 0.000	-0.734, 0.002	-0.794, 0.000	0.717, 0.003	0.852, 0.000
DE	0.726, 0.002	0.628, 0.012	N.S.	-0.604, 0.017	0.517, 0.048	0.664, 0.007
R-1	N.S.	N.S.	N.S.	-0.516, 0.049	N.S.	N.S.
R-2	N.S.	N.S.	0.728, 0.002	-0.727, 0.002	N.S.	-0.515, 0.050
R-3	N.S.	N.S.	-0.765, 0.001	N.S.	N.S.	N.S.
R-4	N.S.	N.S.	-0.768, 0.001	N.S.	N.S.	N.S.
HG	0.667, 0.007	0.760, 0.001	-0.737, 0.002	-0.835, 0.000	0.632, 0.011	0.790, 0.000
RG-I	N.S.	N.S.	N.S.	0.595, 0.017	N.S.	N.S.

*Data are shown as "Pearson correlation, P-value". N.S. = not significant ($p \geq 0.05$).

5.4.2. Water Vapor Barrier Properties

Water vapor permeability (WVP) values of different pectin films are given in Table 5.3. The WVP of films showed a great variation and changed between 6.3 and 31.7 g.mm/m^2.day.kPa. The PSP-Ca^{++} with its 2-to-5-fold lower WVP than those of other films showed the best moisture barrier effect. The PSP, CP-Ca^{++}, and CFP-Ca^{++} films showed intermediate moisture barrier effects, while CSP-Ca^{++}, CP, and AP films showed lower-intermediate, and CFP and CSP showed low moisture barrier effects. It is important to note that the cross-linking did not cause a significant change in the WVP of films obtained from CP and AP ($p > 0.05$). In contrast, CFP, CSP, and PSP films showed 1.7-2-fold higher WVP than their respective cross-linked films ($p \leq 0.05$). These results

suggested that the cross-linking caused formation of denser morphologies for fig pectin films. The WVP values reported in the literature suggested that all pristine fig pectin films developed in the current work showed greater moisture barrier effects than pristine orange and mango peel pectin films (64.7-76.56 g.mm/m^2.day.kPa) (Spatafora Salazar et al. 2019), apple pectin film prepared with pomegranate juice (72 g.mm/m^2.day.kPa) (Azeredo et al. 2016), and pomegranate peel pectin film (60.48 g.mm/m^2.day.kPa) (Oliveira et al. 2016). Moreover, pristine pumpkin pectin film (22.2 g.mm/m^2.day.kPa) (Lalnunthari, Devi, and Badwaik 2020), lime peel pectin film (16.07 g.mm/m^2.day.kPa) (Rodsamran and Sothornvit 2019a), and lemon waste pectin-sweet potato starch blend film (23.76 g.mm/m^2.day.kPa) (Dash et al. 2019) showed better moisture barrier effect than pristine CFP and CSP pectin films, but lower moisture barrier effect than PSP pectin films.

The Pearson's coefficient of correlations (r) suggested that there are significant negative correlations between WVP of pristine films and GA content (r = -0.762) and DE (r = -0.905) of pectins used for film making (Table 5.4) (also see Appendix B as Table B.1 and B.2). These findings suggested that pristine films with good moisture barrier properties need the use of high GA pectins, especially with a high degree of esterification. This result supported the recent finding of Huang et al. (2021), who showed that hydrophobic methyl ester groups in pectins are crucial for the moisture barrier effect of their films. On the other hand, the WVPs of cross-linked films showed a significant negative correlation with DA (r = -0.886) of pectins used in film making. In the literature, it was reported that the high degree of acetylation interfered with the gelation of pectins since the presence of acetyl groups caused steric hindrance for chain association (Vriesmann and Petkowicz 2013). However, it appears that the steric hindrance caused in hydrated pectin molecules by acetyl groups worked differently in dry films. It is well known that the increased degree of acetylation causes a parallel increase in the hydrophobicity of hydrocolloids such as pectin and cellulose since this replaces hydrophilic groups/bonds with hydrophobic acetyl groups (Leroux et al. 2003; Ouarhim et al. 2019). The data in the literature about the effect of acetyl groups in pectins on the WVP of their films are scarce. However, the current work showed for the first time that acetyl groups of fig pectins with DA between 6.95 and 29.9% are highly effective on the WVP of their films when these pectins were cross-linked to form an "egg-box model" configuration. The TPC did not correlate with WVP of pristine films, but it is important

to note that there was a moderately significant negative correlation between the TPC of pectins and the WVP of their cross-linked films (r = -0.745). The hydrophobic interactions formed between aromatic rings (e.g., A and C rings of flavonoids) of polyphenols and hydrophobic methyl groups of pectin are accepted as the primary mechanism of polyphenol-pectin complexation (Liu, Le Bourvellec, and Renard 2020; Tang, Covington, and Hancock 2003). Thus, further studies are needed to understand the effects of possible hydrophobic interactions between polyphenols and acetyl groups of pectins on the WVP of pectin films.

Table 5.3. The water vapor permeability properties of commercial pectin and different fig pectin films.

Film sample	**WVP ($g.mm/m^2.day.kPa$)**	**Film sample**	**WVP ($g.mm/m^2.day.kPa$)**
CP	19.1 ± 1.77^{bc}	**CP-Ca^{++}**	15.0 ± 3.38^{cd}
AP	21.4 ± 6.34^{b}	**AP-Ca^{++}**	20.3 ± 3.46^{b}
CFP	30.4 ± 5.39^{a}	**CFP-Ca^{++}**	17.5 ± 4.55^{bcd}
CSP	31.7 ± 1.42^{a}	**CSP-Ca^{++}**	18.1 ± 2.84^{bc}
PSP	12.9 ± 1.24^{d}	**PSP-Ca^{++}**	6.28 ± 0.57^{e}

*Data are shown as mean ± standard deviation of triplicate measurement. Data at each column indicated by different letters are significantly different ($p \leq 0.05$). -Ca++: denoted for crosslinks for all pectins.

Table 5.4. Correlations between water vapor permeability and molecular properties of commercial pectin and different fig pectin films.

Property		GA	DE	DA	R1	R2	R3	R4	HG	RG-I
WVP (g.mm / m^2.day.kPa)	***Pristine***	-0.762, 0.001	-0.905, 0.035	N.S.	N.S.	N.S.	N.S.	N.S.	-0.679, 0.005	N.S.
	Crosslinked	N.S.	N.S.	-0.886, 0.046	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

*Data are shown as "Pearson correlation, P-value". N.S. = not significant ($p \geq 0.05$).

5.4.3. Solubility and Swelling of Pectin Films

The moisture contents and solubilities of pectin films are shown in Figure 5.1. The moisture contents are varied between 4.07% (CFP-Ca^{++}) and 12.08% (CSP). The pristine films of CP, AP, and PSP showed 100% solubility, while pristine CFP and CSP films showed almost 71-72% solubility due possibly to the crude and heterogeneous nature of their pectins. The cross-linking caused a significant reduction in the solubility of films obtained from CP, CSP, and CFP. Thus, the lowest solubility was obtained for CFP-Ca^{++} (32.8%) followed by CSP-Ca^{++} (38.6%) and CP-Ca^{++} (41.8%). This result clearly showed that the CP, CSP, and CFP pectin contained LMP fractions that turned into insoluble Ca-pectate fractions. In contrast, AP-Ca^{++} and PSP-Ca^{++} showed 100% and 76% solubilities, respectively. The limited reduction in solubility of PSP-Ca^{++} by cross-linking once more suggested that the purification of CSP removed mainly the LMP fractions of PSP pectin. The Pearson's coefficient of correlations (r) suggested that there are highly significant positive correlations between water solubility of pristine films and GA (r = 0.851), DE (r = 0.915) and HG (r = 0.876) of pectins. However, the only significant correlation for cross-linked films was determined between the solubility of these films and the DE of pectins (r = 0.783) used in film making. This finding clearly

showed that the DE of pectins is a very critical factor affecting the solubility of both pristine and cross-linked films.

Due to the high solubility of pristine films, the swelling properties were determined only for the cross-linked films except for that of AP-Ca^{++} which showed 100% solubility (Fig. 6.2). The highest degree of swelling was observed for CP-Ca^{++} followed in descending order by PSP-Ca^{++}, CSP-Ca^{++} and CFP-Ca^{++} films that showed almost 1.9, 2.4, and 3.2-fold less swelling than CP-Ca^{++} film, respectively. Therefore, it is clear that the CFP-Ca^{++} films were not only the least soluble films but also the least swelling films. Due to the limited number of films used in this test, no regression analysis was conducted for film swelling.

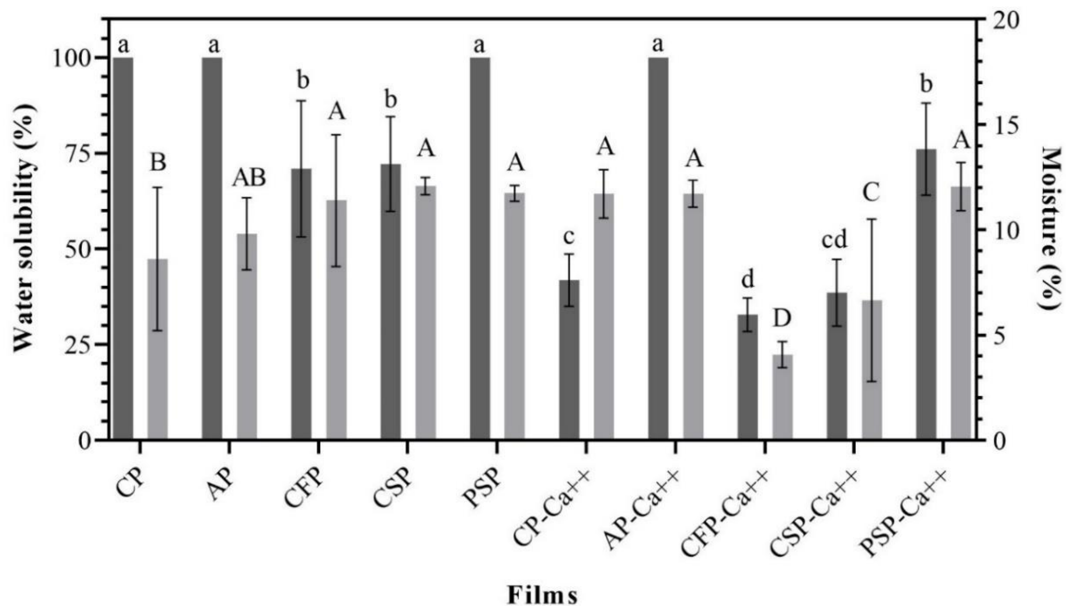

Figure 5.1. Water solubility (dark grey) and moisture (light grey) of commercial pectin and different fig pectin films. Each data point is shown as the average of three replicates. The error bar indicates standard deviation. a-d and A-D at each bar denote a statistically significant difference separately ($p \leq 0.05$). -Ca++: denoted for crosslinks for all pectins.

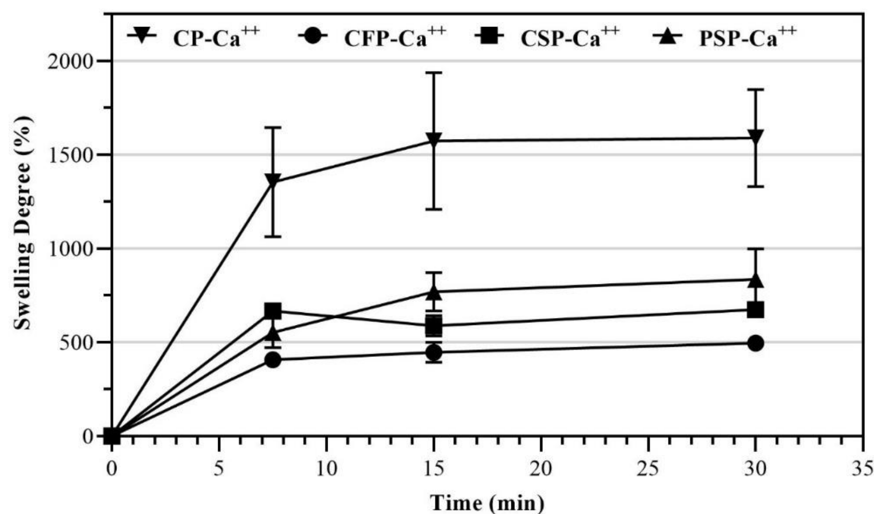

Figure 5.2. The swelling curves of commercial pectin and different fig pectin films. Each data point is shown as the average of three replicates. The error bar indicates standard deviation. -Ca++: denoted for crosslinks for all pectins.

5.4.4. Transparency and Color of Pectin Films

The transparency values of pristine films ranged between 13.8 and 27.2% (Table 5.5). The AP gave the most transparent pristine film followed by pristine films of CP and CSP with intermediate transparency and pristine films of PSP and CFP with low transparency. In all pristine films, the cross-linking caused a significant increase in film transparency ($p \leq 0.05$). The transparency of cross-linked films ranged between 15.8 and 32.6%, but the transparency ranking for the cross-linked films is similar to that of pristine films. According to Hong et al. (2005), the transparency values of polypropylene and low-density polyethylene (LDPE) films were almost 38% and 15–20%, respectively. Thus, it appears that the transparencies of pectin films are comparable to those of LDPE films. It is important to note that CSP and CSP-Ca^{++} films showed significantly greater transparency values than PSP and PSP-Ca^{++} films. This finding clearly showed that the

purification of fig stalk waste pectin did not result in increased film transparency. In general, film transparency is determined by morphology rather than chemical composition (Farris, Introzzi, and Piergiovanni 2009). Thus, it seemed that the difference between the transparency of crude and purified stalk waste pectin films originated from significant differences between their surface (R_{rms} or R_{max}) and cross-sectional morphologies.

The color of films evaluated considering lightness (L*), redness (a*), and yellowness (b*) values and photos of films were given in Table 5.5 and Figure 5.3, respectively. The L* values of films changed between 66.3 and 85.8. The cross-linking did not cause a considerable change in the L* value of films except CP which showed a slight increase in L* by cross-linking. The AP and CP gave the lightest colored films (Fig. 5.3A-D), while all fig pectin films were dark-colored (Fig. 5.3E-J) due to the Maillard reaction products that formed a tight complex with the extracted pectins. However, it must be noted that the purified PSP contained less Maillard reaction products, thus, it gave lighter films than CSP that is a crude stalk waste pectin. The pristine fig pectin films also showed significantly higher a* values than pristine commercial pectin films ($p \leq 0.05$). The cross-linking increased the a* values of films except for pristine PSP films that showed similar a* with PSP-Ca^{++} films. The b* values of fig pectin films were comparable with those of CP films, while AP showed considerably lower b* values than all pectin films.

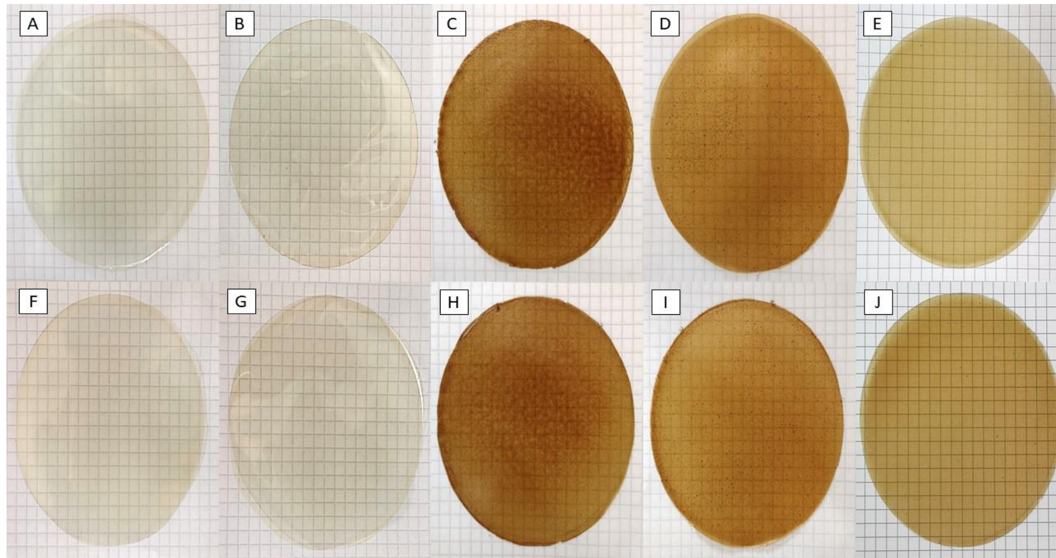

Figure 5.3. Photographic images of commercial pectin and different fig pectin films. The control films; A) CP, B) AP, C) CFP, D) CSP, E) PSP and the crosslinked films; F) CP-Ca^{++}, G) AP-Ca^{++}, H) CFP-Ca^{++}, I) CSP-Ca^{++}, J) PSP-Ca^{++}.

Table 5.5. Transparency (T_{600}) and color values (L*, a* and b*) of commercial pectin and different fig pectin films.

Film sample	T_{600}	L*	a*	b*
CP	21.40 ± 0.17^{e}	77.27 ± 1.32^{c}	-2.68 ± 0.61^{e}	34.85 ± 2.64^{c}
CP-Ca^{++}	24.83 ± 0.11^{c}	79.36 ± 1.03^{b}	1.58 ± 0.26^{d}	25.35 ± 2.11^{e}
AP	27.22 ± 0.02^{b}	85.84 ± 0.83^{a}	-2.96 ± 0.06^{e}	11.6 ± 1.76^{f}
AP-Ca^{++}	32.59 ± 0.01^{a}	85.65 ± 0.2^{a}	1.76 ± 0.07^{d}	8.02 ± 0.58^{g}
CFP	13.76 ± 0.30^{i}	77.77 ± 0.32^{f}	4.05 ± 0.21^{c}	42.18 ± 0.33^{a}
CFP-Ca^{++}	17.34 ± 1.43^{g}	66.33 ± 2.79^{f}	8.48 ± 1.74^{a}	38.65 ± 2.83^{b}
CSP	20.01 ± 0.23^{f}	68.66 ± 0.11^{e}	2.00 ± 0.09^{d}	37.12 ± 0.37^{bc}
CSP-Ca^{++}	23.44 ± 0.09^{d}	70.49 ± 0.44^{de}	5.73 ± 0.15^{b}	31.96 ± 0.61^{d}
PSP	13.77 ± 0.85^{i}	71.78 ± 0.71^{d}	5.15 ± 0.28^{b}	30.78 ± 0.79^{d}
PSP-Ca^{++}	15.79± 0.82^{h}	71.25 ± 0.66^{d}	5.42 ± 0.29^{b}	31.27 ± 0.77^{d}

Data are shown as mean ± standard deviation of triplicate measurement. Data at each column indicated by different letters are significantly different ($p \leq 0.05$). -Ca++: denoted for crosslinks for all pectins. L: Lightness, a*: redness/greenness, b*: yellowness/ blueness.

5.4.5. Morphological Properties of Films by AFM and SEM

The morphologies of films were investigated by AFM and SEM. The surface morphologies (Fig. 5.4A-J) and topographic images (Fig. 5.5A-J) of films obtained by AFM clearly showed that the surfaces of all pectin films were rough. The R_{rms} and R_{max}

of pectin films varied at a broad range between 7.65 and 31.9 nm and 59.9 and 224 nm, respectively (Table 5.6). Considering the roughness parameters, the PSP-Ca^{++} showed the highest roughness followed in descending order by PSP and CSP films that also showed considerable roughness, and CSP-Ca^{++}, CP-Ca^{++}, CP, CFP films with intermediate roughness, and CFP-Ca^{++}, AP, AP-Ca^{++} films with limited roughness. The cross-linking caused some different effects on the surface roughness of pectin films. For example, R_{rms} values of pristine and cross-linked films of AP, CSP, and PSP pectins were similar, while cross-linking caused a significant increase and reduction in R_{rms} values of films obtained from CP and CFP pectins, respectively. Moreover, pristine and cross-linked films of CP, AP, and CFP pectins showed similar R_{max} values, while cross-linking caused an increase and reduction of R_{max} values for films obtained from PSP and CSP pectins, respectively. It is interesting to note that the AP films were the only ones that were not affected by cross-linking.

Figure 5.7A to 5.7J show the SEM micrographs of film surfaces at 500× magnification. The surfaces of pristine and cross-linked films from commercial pectins and CSP pectin were smooth and homogeneous, and they were apparently free from pores, cracks, and air bubbles. The SEM micrographs also proved that the PSP film surface was rough, but it was also evident that these films were apparently free from pores and cracks. In contrast, extensive tiny craters were clearly identifiable on both pristine and cross-linked CFP film surfaces (Fig. 5.7C and 5.7H). Cross-linking improved the surface smoothness of films obtained from commercial pectins and CSP pectin, but no apparent changes were observed in the surface morphologies of CFP and PSP films by cross-linking. Figure 5.8A to 5.8J also shows the cross-sectional SEM images of different pectin films at 2500× magnification. The comparison of cross-sectional images of pristine and cross-linked films indicated that the cross-linking caused formation of extensive networking (intensive tiny aggregations) within films. Some heterogeneous formations were observed in CFP-Ca^{++}, CSP-Ca^{++} and PSP-Ca^{++}, but CP-Ca^{++} and AP-Ca^{++} showed more homogeneous cross-sectional images. No apparent pores and cracks were identified at film cross-sections, except those of CFP and CFP-Ca^{++} films that contained some burst spherical void capsules concentrated mainly at the upper part of the film surface. The CFP pectin showed the highest soluble protein content; thus, these spherical formations might be formed by protein stabilized tiny air bubbles. These results showed that the morphology of all films changed to some extent by cross-linking. Thus, it appears that

the changes in surface roughness and internal morphology together with molecular and compositional parameters determined the final mechanical and barrier properties of cross-linked pectin films. This finding also suggests that some properties of cross-linked films that lack to show any correlation with molecular and compositional parameters of pectins are affected mainly by morphological changes induced by egg-box model formation. Further studies are needed to determine the exact contribution of morphology to mechanical and barrier properties of pectin films.

Table 5.6. Morphological parameters of commercial pectin and different fig pectin films from AFM analysis.

Film sample	R_{rms} (nm)	R_{max} (nm)
CP	10.7 ± 1.34^{d}	84.0 ± 22.9^{de}
CP-Ca^{++}	16.1 ± 1.36^{bc}	123.9 ± 23.1^{cd}
AP	6.72 ± 1.81^{e}	68.2 ± 32.2^{e}
AP-Ca^{++}	7.72 ± 3.15^{e}	62.1 ± 13.8^{e}
CFP	11.7 ± 2.82^{cd}	82.7 ± 18.1^{de}
CFP-Ca^{++}	7.65 ± 2.18^{e}	59.9 ± 14.2^{e}
CSP	19.8 ± 6.16^{b}	177 ± 78.5^{ab}
CSP- Ca^{++}	16.4 ± 3.81^{bc}	118 ± 19.5^{cd}
PSP	22.8 ± 3.14^{ab}	157 ± 22.0^{bc}
PSP-Ca^{++}	31.9 ± 5.49^{a}	224 ± 27.4^{a}

*Data are shown as mean ± standard deviation of at least four measurements. Values at each column indicated by different letters are significantly different ($p \leq 0.05$). -Ca++: denoted for crosslinks for all pectins.

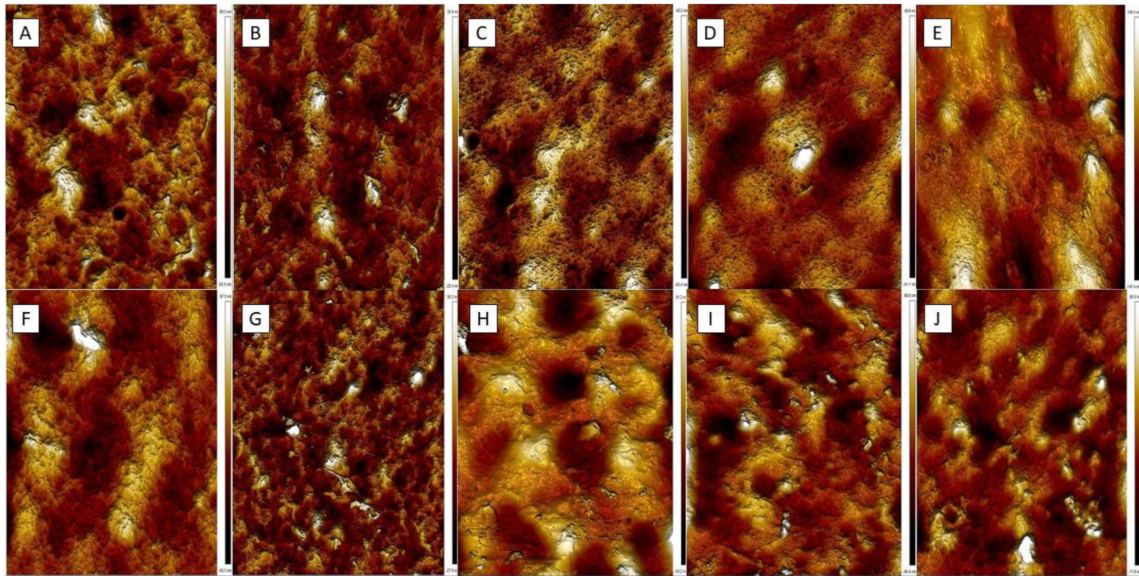

Figure 5.4. The surface morphologies of commercial pectin and different fig pectin films: (A) CP; (B) AP; (C) CFP; (D) CSP; E) PSP; (F) CP-Ca^{++}; (G) AP-Ca^{++}; (H) CFP-Ca^{++}; (I) CSP-Ca^{++}; and J) PSP-Ca^{++}.

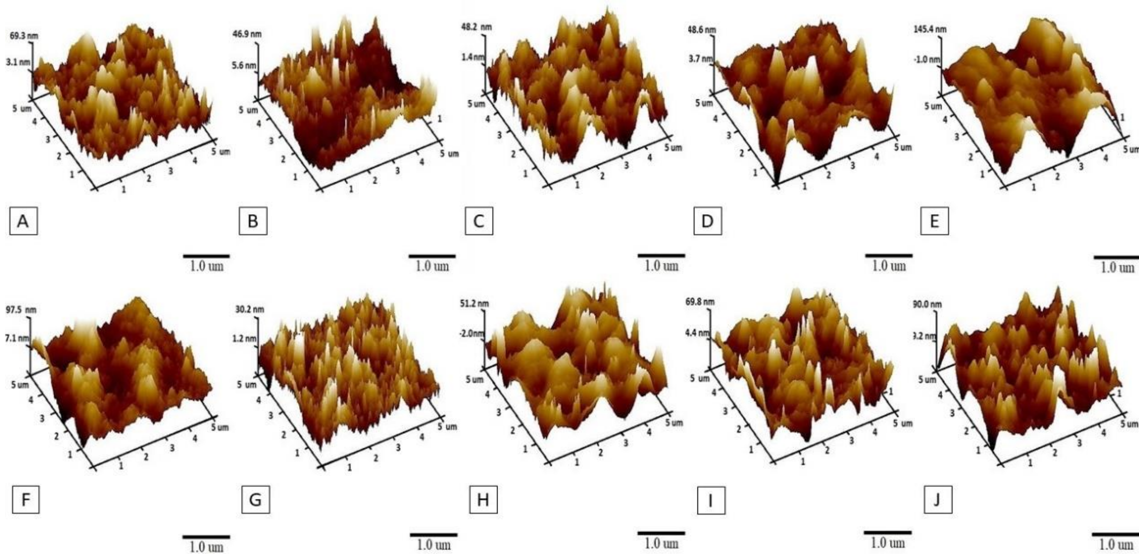

Figure 5.5. The topographic images of commercial pectin and different fig pectin films: (A) CP; (B) AP; (C) CFP; (D) CSP; E) PSP; (F) CP-Ca^{++}; (G) AP-Ca^{++}; (H) CFP-Ca^{++}; (I) CSP-Ca^{++}; and J) PSP-Ca^{++}.

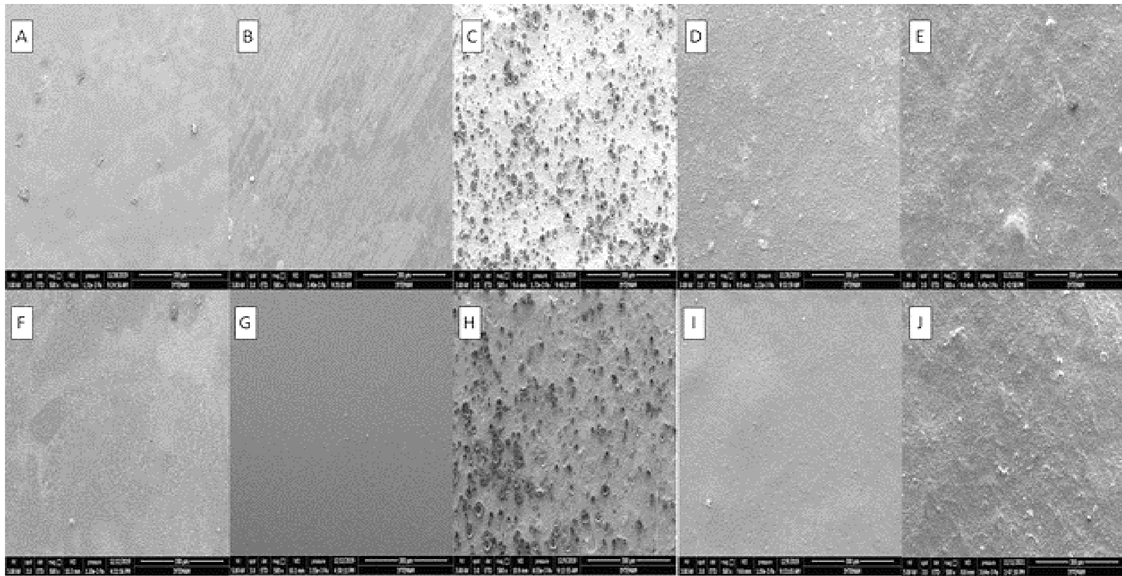

Figure 5.6. The surface morphology of commercial pectin and different fig pectin films: (A) CP; (B) AP; (C) CFP; (D) CSP; E) PSP; (F) CP-Ca^{++}; (G) AP-Ca^{++}; (H) CFP-Ca^{++}; (I) CSP-Ca^{++}; and J) PSP-Ca^{++}. (Magnification: 500×, Scale bar: 300μm).

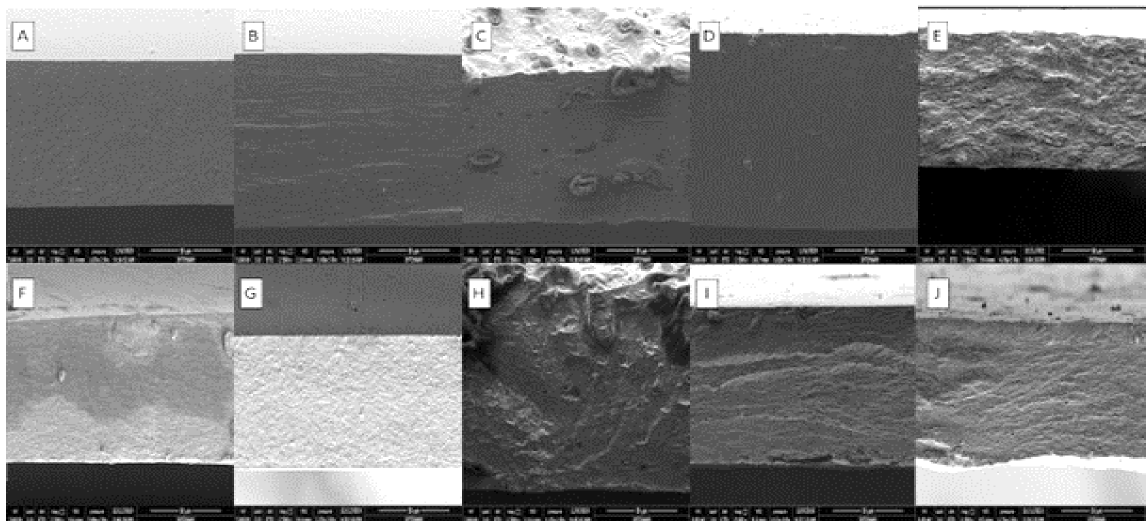

Figure 5.7. The cross-sectional morphology of commercial pectin and different fig pectin films: (A) CP; (B) AP; (C) CFP; (D) CSP; E) PSP; (F) CP-Ca^{++}; (G) AP-Ca^{++}; (H) CFP-Ca^{++}; (I) CSP-Ca^{++}; and J) PSP-Ca^{++}. (Magnification: 2500×, Scale bar: 50μm).

5.5. Conclusions

This work clearly showed the potential advantages of using pectins extracted from stalk waste of processed high-quality figs in the development of edible films. Edible films of purified stalk waste pectin showed superior mechanical strength than commercial apple pectin while having comparable mechanical strength with commercial citrus pectin. The pristine and cross-linked films of purified stalk waste pectin had the highest moisture barrier effects. The films of pectin extracted from low-grade substandard fig fruits did not show outstanding mechanical and barrier properties, but the cross-linked films of this pectin showed the highest surface hydrophobicity and lowest solubility and swelling. The analysis of Pearson's coefficient of correlations revealed fundamental knowledge about the effects of molecular and compositional parameters of studied pectins on the properties of their pristine and $CaCl_2$ cross-linked films. The major findings are as follows: (1) galacturonic acid content of pectins is the primary factor correlating positively with mechanical strength and stiffness of pristine and cross-linked films, (2) the moisture barrier effect of pristine films correlates with high galacturonic acid content and high degree of esterification while moisture barrier effect of cross-linked films correlates with high degree of acetylation, (3) the phenolic content of pectins correlates negatively with moisture barrier effect of cross-linked films. This work not only introduced fig stalk pectin as an alternative hydrocolloid that gives some superior edible film characteristics than commercial pectins, but also expanded the fundamental knowledge about factors affecting the mechanical and barrier properties of pectin films.

CHAPTER 6

DEVELOPMENT AND CHARACTERIZATION OF EMULSION BASED EDIBLE FILM FROM FIG PECTIN

6.1. Introduction

The microbial outbreaks originating from fruits that grow on the ground are observed very frequently, as they are in direct contact with potential microbial contaminants such as irrigation water, sewage, manure or fertilizer, and animals (Sapers and Sites 2003; Chen et al. 2012; Ma et al. 2016; De Corato 2019). The melons are among the most important risk fruits since their stem scar and rough peel provide a unique protective environment for pathogenic bacteria such as Listeria monocytogenes, Salmonella enterica and Escherichia coli O157:H7 (Chen et al. 2012; Sapers and Sites 2003) that could easily contaminate the inner edible parts of fruit during processes such as cutting and slicing (Ma et al. 2016). The cantaloupe (muskmelon, mushmelon, rockmelon or sweet melon) is a particularly risky melon cultivar since it has a complex webbed rind surface that provides a protective growth medium for the Listeria spp. (Behrsing et al. 2003). An outbreak of listeriosis in the United States of America linked to cantaloupe melons in 2011 clearly proved the great risk of webbed rind melons since it caused 33 deaths, 1 miscarriage and 143 hospitalizations (CDC 2012). In 2018, consumption of L. monocytogenes-contaminated cantaloupes caused 7 deaths, 1 miscarriage with a total of 22 confirmed cases in Australia (NSW DPI 2018).

Active edible coatings incorporated with antimicrobials have been increasingly employed to inhibit microbial pathogens and to increase the quality of fresh-cut fruits (Rojas-Graü et al. 2009). Edible films of chitosan, alginate and zein incorporated with different natural and chemical agents (e.g., cinnamon oil, allyl isothiocyanate, eugenol, nisin, lauric arginate, ethylenediaminetetraacetic acid) have been applied for coating of whole melons (Ma et al. 2016; Chen et al. 2012; Boyacı et al. 2019). However, studies related to the application of emulsion-based pectin coatings incorporated with natural antimicrobials for coating of whole melons are scarce.

The objective of the current chapter is to employ antimicrobial emulsion-based coatings of pectin from different sources with EUG to eliminate *Listeria* contaminated on Galia melons (hybrids of cantaloupe and honeydew melons), which have a complex webbed rind surface (as cantaloupe) and a sweet, creamy textured, light yellow to green flesh (as honeydew). Due to its inherent bio-adhesive properties (Farris et al. 2011), pectin might be a suitable coating material to deliver antimicrobials onto fruit surface contaminated with pathogens. The main original feature of this study is that, for the first time, it proposed the development of edible coatings using pectin extracted from wastes arising from the industry of sun-dried fig processing. This work opens new perspectives to fruit industry by employing sun-dried fig processing wastes in production of a value-added product such as pectin, and by characterization and application of obtained pectin films for coating of webbed-rind melons that cause challenging safety problems. The coating procedure developed in this work could be applied to melons at the post-harvest period in packaging houses following classical washing procedures. Such an antimicrobial coating procedure is an additional measure against remaining pathogens at the fruit surface. Besides, some of the results presented in this chapter were already published by Çavdaroğlu, Farris, and Yemenicioğlu (2020).

6.2. Materials

Citrus pectin (P9135, 79% GA) and eugenol (E51791) were obtained from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals were reagent grade. Purees of sun-dried fig processing wastes (a mixture of highly defected fruits, fruit residues from processing, fruits routinely separated for quality control), all passed from UV inspection for luminescence that indicates aflatoxins, were kindly supplied by KFC Gıda A.S. (İzmir, Turkey). The purees were mixed, divided into small portions, and kept at -20 °C until used for pectin extraction. The *Listeria innocua* NRRL-B 33314 (ATCC 1915) was from the culture collection of the microbiology laboratory of the Department of Food Engineering at İzmir Institute of Technology. The Galia melons (*Cucumis melo* var. *reticulatus*) were purchased from a local market in İzmir, Turkey.

6.3. Methods

The methods given below were performed to develop and characterize the emulsion based edible film forming properties of fig pectins with the presence of eugenol and apply as antimicrobial coating to the webbed melon surfaces against *L. innocua*.

6.3.1. Acidic Extraction and Characterization of Molecular Properties of Fig Pectin

Purees of sun-dried fig processing wastes were subjected to acid extraction in this part of the study as described in Section 3.3.1. In the current chapter, to minimize modifications in pectin chain length, mild extraction at 3% (w/v) CA for 1 h was applied rather than extraction at 6% (w/v) CA for 1 h. The molecular properties of fig pectin were characterized as in Section 3.4.4.

6.3.2. Preparation of Pectin-EUG Emulsion-Based Films and Coatings

For this purpose, 3 g of CFP or CP was suspended in 100 mL of distilled water. The suspension was then heated on a hotplate under continuous stirring at 60 °C for 30 minutes. After cooling to room temperature, the solution was homogenized at 10,000 rpm for 1 minute using a homogenizer (Heidolph, Germany, rotor ϕ = 6.6 mm tip). The pH of the mixture was adjusted to 8.0 using 4 M NaOH, and it was stirred for 30 minutes to cause de-esterification of pectin. This process intended increasing pectins' negatively charged exposed –COO- groups that are important to create repulsive forces among oil droplets and increase emulsion stability (Ngouémazong et al. 2015). The pH of the mixture was then slowly lowered to a final value between 3 and 4 with 1 M HCl. After that, 0.9 g of glycerol (30% of pectin, w/w) was added as a plasticizer of emulsion films, and the mixture was stirred for 15 minutes. Finally, to prepare CFP-EUG and CP-EUG emulsions, EUG was added into the mixture at different concentrations (0.25%, 0.5%, 1% or 2%, (w/w)), and the mixtures were then homogenized at 10,000 rpm for 4 min. The freshly prepared emulsions with desired amounts of EUG were used directly in melon coating studies as described in section 6.3.5. On the other hand, films used in

characterization studies and zone inhibition tests on agar surface were obtained by the casting method. To obtain pre-cast films, 20 g of emulsion was cast onto a glass Petri dish (inner diameter 10 cm), which was dried in a controlled test cabinet at 25 °C and 50% RH for 24 h.

6.3.3. Stability of Pectin-EUG Emulsions

The stability of CFP-EUG and CP-EUG emulsions was determined by monitoring the turbidity evolution for 10 days at 10 °C. The turbidity values were expressed in nephelometric turbidity units (NTU) using a HACH turbidity meter (2100 AN, USA) and in absorbance units by measuring the absorbance of emulsions at 600 nm. Tests were conducted using two replicates. Zeta potential and particle size of the emulsions were also determined using a NanoPlus DLS Particulate Systems (Micromeritics Instrument Corporation, GA, US).

6.3.4. Antimicrobial Activity of Films on Inoculated Agar Surface

The antimicrobial activity of freshly prepared pre-cast CFP-EUG and CP-EUG films containing EUG between 0.25% and 2% (w/w) were tested under aseptic conditions by the agar diffusion method as reported by Boyacı et al. (2019) using *L. innocua* as test microorganism. The discs (diameter: 1.3 cm) of pre-cast and dried films were formed at aseptic conditions by a cork borer. Antimicrobial activity test was repeated twice for each film by using total of 18 discs (1 disc was placed per Petri dish) from each film type. The

diameters of the clear zones formed around the discs were measured by a micrometer (Chronos®, UK) and the average zone areas were expressed in cm^2.

6.3.5. Antimicrobial Activity of Coatings on Inoculated Webbed-Rind Melons

The antimicrobial activity of CFP-EUG and CP-EUG coatings with 2% EUG was also tested on inoculated melons. Melons were first washed extensively in tap water, followed by ethanol (70%, w/w) and sterile distilled water. The cleaned melons were then left to dry under the laminar flow hood overnight at room temperature. For the preparation of the inoculum, overnight cultures of *L. innocua* grown in nutrient broth under aerobic conditions at 37 °C were prepared. One mL of this active culture was transferred to 9 mL of nutrient broth in a tube and incubated at 10 °C for 24 h to promote the adaptation of the culture to the cold-storage conditions. Two separate zones (4 cm × 4 cm) on each melon's surface were then inoculated by spreading 150 µL of the *L. innocua* culture (10^8 CFU/mL). The inoculated melons were kept under aseptic conditions for 20 min to promote the absorption and drying of the inoculum on the melon surface. Freshly prepared solutions of CFP-EUG or CP-EUG containing EUG at 2% (w/w) and control CFP or CP pectin film solutions (150 µL) were then pipetted onto the inoculated areas (4 cm × 4 cm) of melons and spread homogeneously using a sterile plastic rod. Inoculated melons without film treatment were used as control. Melons were kept for 30 min under laminar flow hood to dry pectin film solutions on their surface (0th day). After that, melons were stored at 10 °C and 50% RH for 7 days and enumerated for their *L. innocua* counts.

Microbiological tests were carried out on the 0th and 7th days. A 10 g portion of treated areas (4 cm × 4 cm area framed previously with a marker) of melon rind were excised using a sterile knife. The cut rind pieces were placed into stomacher bags (Thermo Fisher Scientific, Inc., Waltham, MA) containing 90 mL sterile 0.1% (w/w) peptone water and homogenized for 150 s using a stomacher (BagMixer ® 400, Interscience, France). The homogenates were then serially diluted with 0.1% w/v peptone water and surface

plated on Oxford Listeria Selective Agar (Merck, Darmstad, Germany) with Oxford Listeria Selective Supplement (Merck, Darmstad, Germany). Counting of colonies was carried out after 48-h incubation at 37 °C. The counts were performed on triplicate plates for 2 inoculated areas (4 cm × 4 cm) for each treatment (uncoated, CFP or CP coated, and CFP-EUG or CP-EUG coated samples). Microbiological counts were expressed as logarithm (Log) of colony-forming unit per gram (Log CFU/g) for each treatment.

6.3.6. Mechanical Tests

Tensile strength at break (TS), elongation at break (EAB), and Young's modulus were determined using Texture Analyzer TA-XT2 (Stable Microsystems, Godalming, UK) according to ASTM Standard Method D882-02 (ASTM 2002a). The dried films were conditioned in a controlled test cabinet at 25 °C, 50% RH for 24 h before testing. Then, the films were cut into 50-mm-long and 8-mm-wide strips. The initial grip distance was 50 mm and the drawing speed was 50 mm / min. The average of eight measurements was taken. At least eight strips of each film were tested, and films prepared with duplicated. The thickness of films was measured by using a micrometer (Chronos, UK).

6.3.7. Morphology of Film

The cross-sectional morphologies of pectin film samples with and without eugenol were examined by using SEM (250 Quanta FEG, FEI Company, United States). The films were prepared by crushing them after freezing in liquid nitrogen. Specimens were gold-coated with a sputter coater (Emitech K550X, Quorum Technologies Inc.UK) under 15 mA for 60 s.

6.3.8. Statistical Analysis

Statistical difference between treatments was determined by using variance analysis (one way-ANOVA) and Fisher post-test ($p \leq 0.05$) using Minitab (ver.18.1, Minitab Inc., United Kingdom).

6.4. Result and Discussion

The detailed results and discussion will be given in the following sections.

6.4.1. Stability of Pectin-EUG Emulsions

The 3% (w/v) solutions of CFP or CP formed highly turbid and stable emulsions with EUG at concentrations between 0.25 and 2% (w/w) as shown in Figure 6.1. This was demonstrated by the stable spectrophotometric absorbance measurements at 600 nm for both CFP-EUG and CP-EUG emulsions, that cold-stored at 10 °C for 10 days (Table 6.1). The turbidity of all CFP-EUG and most of CP-EUG emulsions were also not measurable (> 10000 NTU) (Table 6.1). The only measurable NTUs were those obtained from CP at 0.25% EUG and 1% EUG, with values that remained stable during 10 days of cold storage in the ranges 4000-5000 NTU and 8000-10000 NTU, respectively (Table 6.1). The stability of CFP-EUG and CP-EUG emulsions were also proved by their limited changes

in droplet size and zeta potential values during 10 days of cold storage (Table 6.2). The initial droplet size of CFP-EUG emulsions was significantly lower than that of CP-EUG emulsions. This result seems to indicate a better emulsifying capability of CFP than CP, which could be ascribed to the high protein content of CFP coming from the seeds of fig (CFP and CP contain 15.0 and 6.2 g protein/100 g pectin, respectively). No significant differences were detected in the droplet size (3.46 to 10.08 μm) for both types of emulsions between 3 and 10 days of cold storage, which suggests that CFP and CP behaved in a similar way in terms of emulsion stability. It is also important to note that both emulsions remained stable, and they did not show any phase separation of a minimum of 6 weeks at 4 °C. Moreover, CFP-EUG emulsions showed slightly to moderately lower zeta potential values than CP-EUG emulsions, possibly due to a lower GA content (means less –COO-), but higher protein content (might mask negative charges) of CFP than CP. Further long-term storage stability tests are needed to understand the feasibility of commercializing ready-to-use emulsion preparations. However, it should be noted that the best performance (homogeneity and effectiveness) from antimicrobial emulsion-based coatings with volatile essential oils are obtained when their emulsions are prepared freshly before each application.

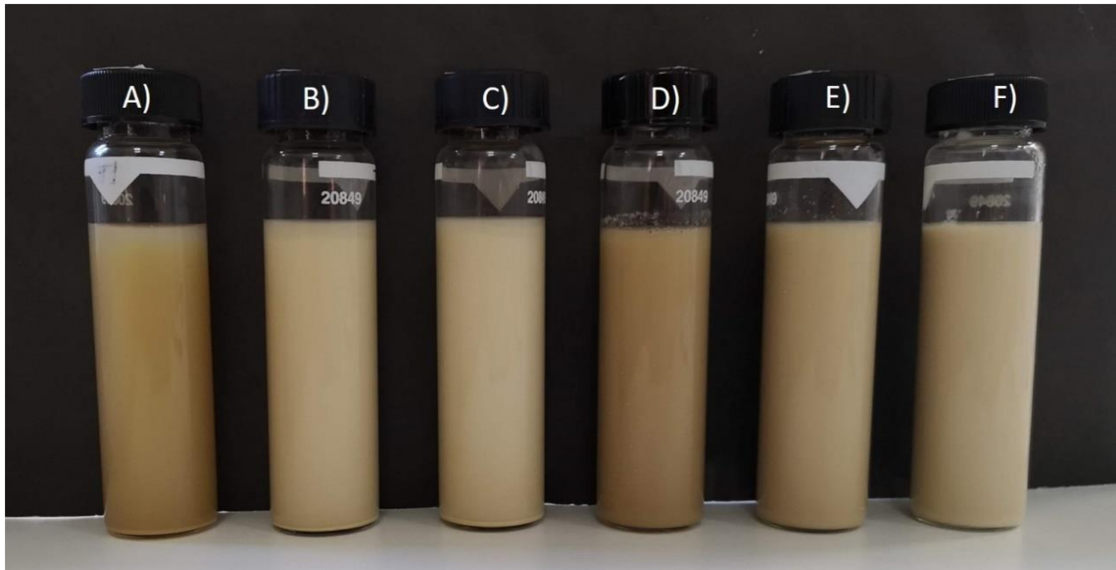

Figure 6.1. Photographic images of pectin-eugenol emulsions; (A) CFP-EUG (0.25%, w/w), (B) CFP-EUG (1 %, w/w), (C) CFP-EUG (2%, w/w), (D) CP-EUG (0.25%, w/w), (E) CP-EUG (1%, w/w), (F) CP-EUG (2%, w/w).

Table 6.1. Turbidity values of CFP-EUG and CP-EUG emulsions during cold storage at 10 °C.

EUG (%, w/w)	Spectrophotometer at 600 nm			
	0th Day	**3rd Day**	**7th Day**	**10th Day**
CFP-EUG emulsions				
0.25	2.52 ± 0.00 ^{b,AB}	2.55 ± 0.01 ^{b,A}	2.52 ± 0.01 ^{c,B}	2.53 ± 0.01 ^{c,AB}
1	2.53 ± 0.01 ^{ab,B}	2.57 ± 0.00 $^{a.A}$	2.54 ± 0.01 ^{b,B}	2.55 ± 0.01 ^{b,AB}
2	2.54 ± 0.01 ^{a,C}	2.57 ± 0.00 ^{a,AB}	2.56 ± 0.00 ^{a,B}	2.58 ± 0.00 ^{a,A}
CP-EUG emulsions				
0.25	2.41 ± 0.00 ^{c,A}	2.38 ± 0.00 $^{c.B}$	2.36 ± 0.00 ^{e,C}	2.38 ± 0.00 ^{e,B}
1	2.53 ± 0.00 $^{ab.A}$	2.53 ± 0.00 $^{b.A}$	2.49 ± 0.01 ^{d,C}	2.50 ± 0.01 ^{d,B}
2	2.55 ± 0.01 $^{a.B}$	2.57 ± 0.00 $^{a.A}$	2.53 ± 0.00 ^{b,C}	2.55 ± 0.00 ^{b,B}

EUG (%, w/w)	Turbidimeter, NTU			
	0th Day	**3rd Day**	**7th Day**	**10th Day**
CFP-EUG emulsions				
0.25	>9999 NTU	>9999 NTU	>9999 NTU	>9999 NTU
1	>9999 NTU	>9999 NTU	>9999 NTU	>9999 NTU
2	>9999 NTU	>9999 NTU	>9999 NTU	>9999 NTU
CP-EUG emulsions				
0.25	4328 ± 29.0 ^{c,C}	4964 ± 32.5 $^{a.A}$	4946 ± 27.6 $^{a.A}$	4700 ± 65.8 $^{b.B}$
1	9070 ± 110 $^{ab.AB}$	9103 ± 11.3 $^{ab.AB}$	9496 ± 395 $^{a.A}$	8802 ± 46.0 $^{b.B}$
2	>9999 NTU	>9999 NTU	>9999 NTU	>9999 NTU

* Data are shown as mean ± standard deviation of triplicate measurement. a-e and A-C values at each column and row followed by different letters indicate statistically significant differences analyzed individually in triplicate ($p \leq 0.05$), respectively.

Table 6.2. Droplet sizes of CFP-EUG and CP-EUG emulsions during cold storage at 10 °C.

EUG (%, w/w)	D[3,2], µm			
	0th Day	3rd Day	7th Day	10th Day
CFP-EUG emulsions				
0.25	1.99 ±0.28 c,C	3.46 ±0.07 d,B	3.52 ±0.83 c,B	4.65 ±0.32 c,A
1	2.02 ±0.29 c,B	3.89 ±0.09 d,A	3.91 ±0.72 c,A	4.52 ±0.87 c,A
2	2.31 ±0.64 c,B	4.19 ±0.38 d,A	3.96 ±0.44 c,A	4.55 ±0.51 c,A
CP-EUG emulsions				
0.25	6.93 ±0.4 b,AB	7.15 ±0.36 c,A	6.60 ±0.82 b,AB	6.11 ±0.98 b,B
1	8.41 ±3.08 b,A	8.96 ±1.87 b,A	6.92 ±1.12 b,A	7.18 ±1.19ab,A
2	11.2 ±2.76 a,A	10.1 ±0.75 a,AB	8.99 ±1.61 a,AB	8.70 ±1.70 a,B

EUG (%, w/w)	Zeta Potential, mV			
	0th Day	3rd Day	7th Day	10th Day
CFP-EUG emulsions				
0.25	-9.48 ± 1.22 c,A	-9.57 ±0.80 bc,A	-10.9 ±5.40 b,AB	-15.0 ±1.19 b,B
1	-5.64 ± 0.77 b,A	-6.17 ±0.00 ab,A	-5.65 ±0.00 a,A	-5.35 ±0.89 a,A
2	-2.12 ± 0.49 a,A	-3.34 ±0.57 a,A	-6.51 ±1.21 ab,B	-7.50 ±1.67 a,B
CP-EUG emulsions				
0.25	-17.3 ±0.28 d,B	-16.2 ±0.21 d,A	-16.4 ±0.27 c,A	-16.4 ±0.24 b,A
1	-19.1 ±2.79 d,A	-20.1 ±3.80 e,A	-19.2 ±2.73 c,A	-20.3 ±4.10 c,A
2	-10.3 ±2.51 c,A	-13.0 ±2.85 cd,AB	-16.3 ±1.15 c,BC	-18.3 ±2.42 bc,C

* Data are shown as mean ± standard deviation of triplicate measurement. a-e and A-C values at each row and column followed by different letters indicate statistically significant differences analyzed individually in triplicate ($p \leq 0.05$), respectively.

6.4.2. Antilisterial Activity of Films in Zone Inhibition Test

The photographs of inhibition zones are displayed in Figure 6.2 and Figure 6.3 while overall results of the zone inhibition tests with films containing EUG between 0.25% and 2% against *L. innocua* are displayed in Figure 6.4. The minimum amounts of EUG (w/w) in films that yielded clear zones around the discs of CFP and CP films were 0.25% and 0.5%, respectively. However, the clear zones at the indicated EUG concentrations were observed only in 8 out of 18, and 14 out of 18 discs tested for CFP and CP films, respectively, due to the fact that the inhibitory concentration was not reached in a homogeneous manner all around the discs. However, the 'no zone discs' were eliminated when EUG concentrations were increased. The CFP-EUG films formed significantly greater zone areas (5 and 1.6-fold) than CP-EUG films at 0.5% and 1% EUG concentrations, respectively. Thus, it is clear that CFP-EUG emulsion-based films performed better than CP-EUG films in terms of EUG release at the test conditions used in the experiment. However, both CP-EUG and CFP-EUG films showed similar antimicrobial activities when the concentration of the essential oil was increased to 2% (w/w), a critical concentration that provided an excessive amount of EUG in both films for effective inactivation of *Listeria*. Thus, the optimal EUG concentration of 2% (w/w) was used in films tested on inoculated melons.

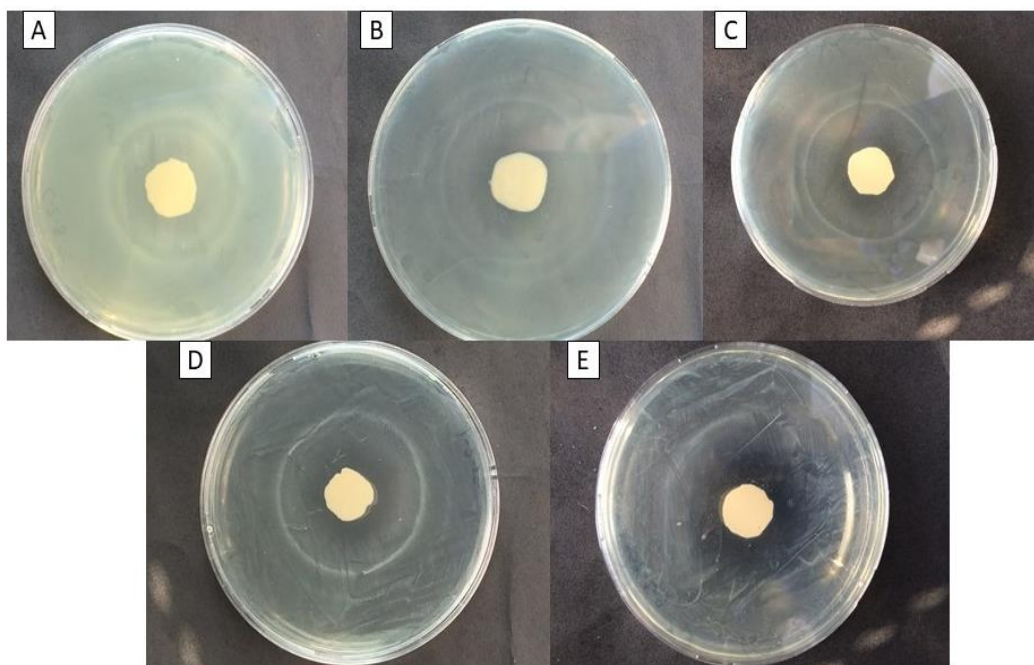

Figure 6.2. Zone inhibition photos of (A) CFP control, (B) CFP-EUG (0.25%, w/w), (C) CFP-EUG (0.5%, w/w), (D) CFP-EUG (1%, w/w), (E) CFP-EUG (2%, w/w) on *L. innocua.*

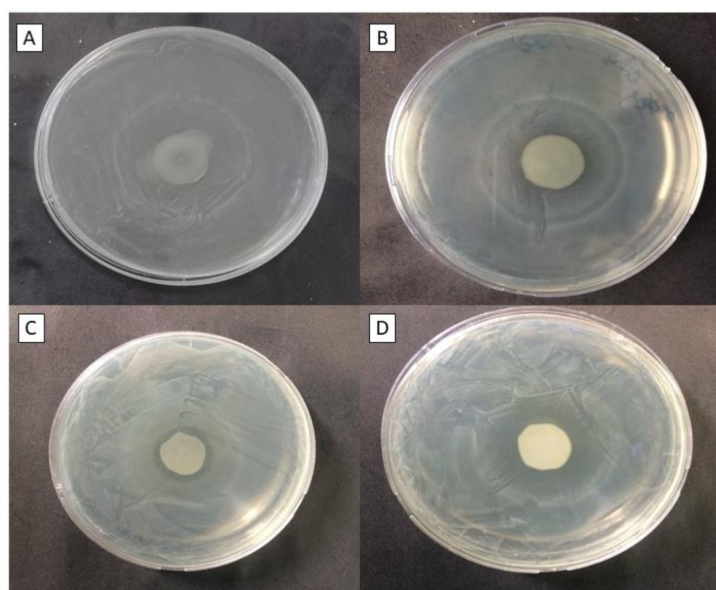

Figure 6.3. Zone inhibition photos of (A) CP control, (B) CP-EUG (0.5%, w/w), (C) CP-EUG (1%, w/w), (D) CP-EUG (2%, w/w) on *L. innocua.*

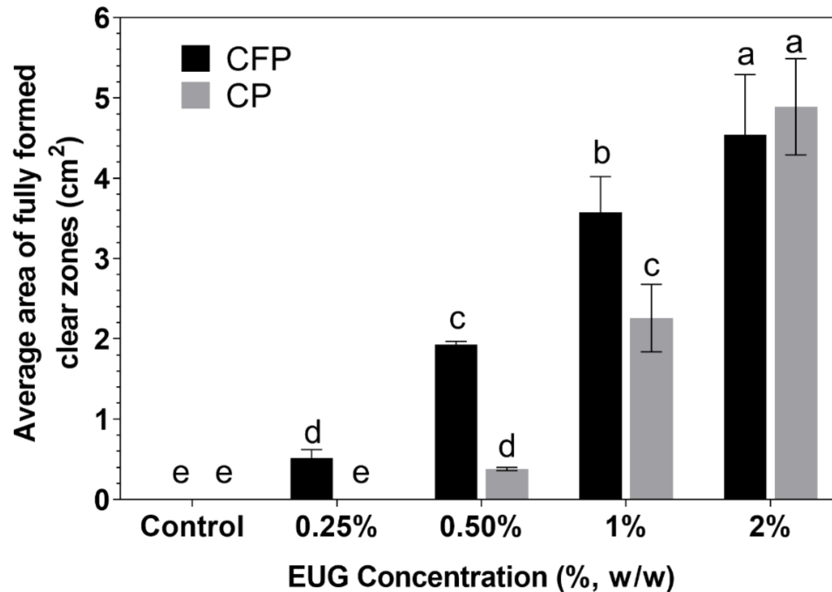

Figure 6.4. Zone-inhibition based antimicrobial activity of CFP-EUG and CP-EUG films on *L. innocua*. Each data point is shown as the average of two replicates. The error bar indicates standard deviation. Data with different letters are significantly different ($p \leq 0.05$).

6.4.3. Antilisterial Activity of Films at Inoculated Webbed Melon Surfaces

The application photos of selected pectin emulsion coatings on melon surfaces are shown in Figure 6.5 while the overall results of *L. innocua* counts conducted at the beginning and at the end of 1-week cold storage (at 10 °C) for uncoated, and CFP, CP, CFP-EUG, and CP-EUG coated inoculated webbed melons are presented in Figure 6.6. The listerial counts of uncoated control melons and control CP coated melons were not significantly different at 0th day ($p > 0.05$) while CP coated melons showed significantly lower listerial counts at 7th day of cold storage ($p \leq 0.05$). In contrast, it is important to note that melons coated with control CFP films gave significantly lower Listerial counts than uncoated controls at both 0th and 7th days of cold storage ($p \leq 0.05$). This finding

suggested that the pectin extracted from sun-dried fig wastes showed an inherent antimicrobial activity that might originate from the antimicrobial activity of polyphenols associated with this hydrocolloid (Amessis-Ouchemoukh et al. 2017). Polysaccharides like pectin interact with polyphenols via their polar groups (e.g. acetal, hydroxyl or carboxyl) and bind phenolic –OH groups with H-bonds and van der Waals forces (Palafox-Carlos, Ayala-Zavala, and González-Aguilar 2011). Thus, polyphenols could not be separated effectively from pectin by classical ethanol precipitation and washing cycles applied during pectin purification. Gharibzahedi et al. (2019) reported that pectin from peels of fresh common figs contained 3 g GAE equivalents of total phenols per 100 g of pectin (d.w.). In the current study, the total phenolic content of extracted CFP was almost 2-fold higher than that of CP (as 1.03 and 0.55 g GAE/100 g of pectin (d.w.), respectively). Thus, these results supported that the inherent antimicrobial activity of CFP control film could be due to its high polyphenol content. At the same time, the antimicrobial activity on coated melons against *L. innocua* increased significantly by application of pectin-EUG coatings. It is important to note that on the 0th day, the listerial counts of melon surfaces coated with CP-EUG and CFP-EUG films were 1.5 and 1.3 decimals (D) lower than the listerial counts obtained from uncoated melon surfaces, respectively. Moreover, the decimal differences between *Listeria* counts of uncoated and CP-EUG or CFP-EUG film-coated samples increased to 2.7 and 2.2 D by 1-week cold storage, respectively. Although the listerial counts of melons coated with CP-EUG were statistically significantly ($p \leq 0.05$) lower than those coated with CFP-EUG at the end of 7th days, the antimicrobial performance on webbed-rind melon surfaces of the pectin coatings from different origin was comparable. After 1-weeks storage, the sharp smell of EUG in melons with CP-EUG and CFP-EUG coatings reduced considerably due to evaporation of essential oil from the pectin films. Thus, it seemed that the increased decimal reduction in *Listeria* by storage was related mainly with death of bacteria damaged at the initial stages of coating when pectin films had contained high concentrations of EUG. Further studies are needed to determine the kinetics of EUG loss from both types of pectin films at different temperatures, and to understand duration of antimicrobial effectiveness following application of films.

In the literature, studies related to the use of pectin coatings with essential oils on whole webbed rind melons are scarce. However, Boyacı et al (2019) reported that zein films with 2% (w/w) EUG caused almost 3.3 D reduction in listerial counts of some melon cultivars (Santa Claus and Crenshaw) having smooth to slightly rough rind surface within

1-week of cold storage. This result indicated the possibility that the pectin emulsion coatings with EUG are slightly less effective on whole melon surfaces than zein coatings with EUG. However, both films should be compared for the same film thickness on webbed rind melons that provide a better hiding and growth medium for Listeria than smooth rind melon cultivars (Behrsing et al. 2003). On the other hand, Ma et al. (2016) obtained > 3 log CFU/cm^2 reduction of *Escherichia coli* O157:H7 and *Listeria monocytogenes* on whole cantaloupes immediately after applying chitosan coating with 0.1% lauric arginate, 0.1% ethylenediaminetetraacetic acid and 1% cinnamon oil on their surface. Moreover, Zhang et al. (2015) also achieved effective inhibition of different pathogens including *Listeria monocytogenes* on cantaloupes using alginate films with 2% cinnamon bark oil. All these studies clearly showed the good potential of using antimicrobial coatings to increase the safety of whole webbed rind melons. However, further studies are needed to determine long term effects of edible coatings on the quality attributes (e.g., surface color, texture and sensory properties) of melons.

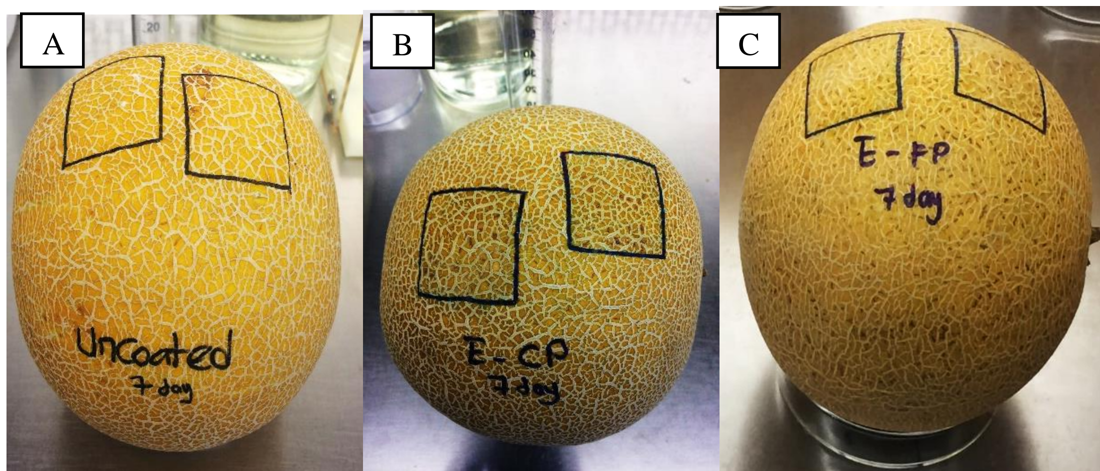

Figure 6.5. Photographic images of (A) uncoated, (B) CP-EUG (2%, w/w) coated, (C) CFP-EUG (2%, w/w) coated Galia melons.

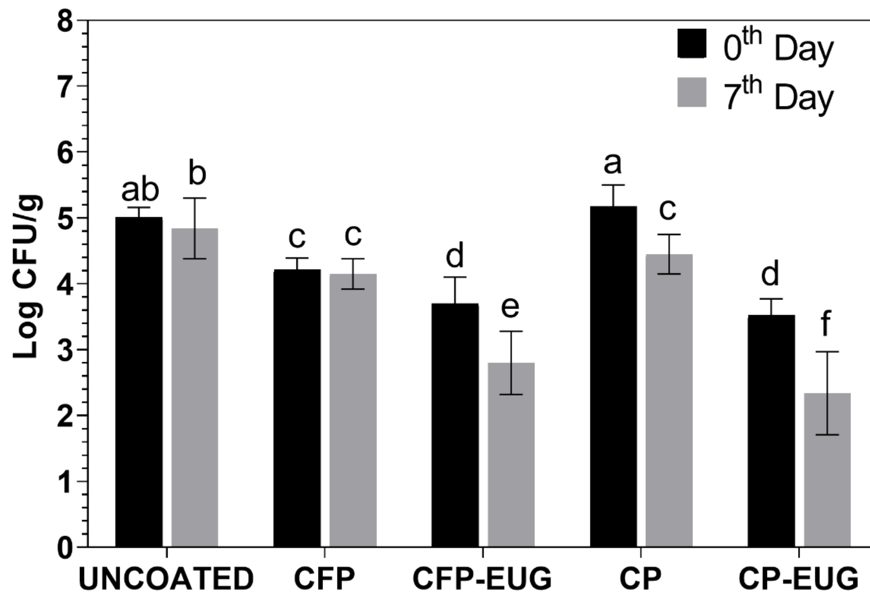

Figure 6.6. Antimicrobial effects of CFP-EUG and CP-EUG coatings with 2% (w/w) EUG on *L. innocua* inoculated onto whole Galia melons during cold storage at 10 °C. Each data point is shown as the average of two replicates. The error bar indicates standard deviation. Data with different letters are significantly different ($p \leq 0.05$).

6.4.4. Mechanical Properties of Films

The photographs and the mechanical properties of films obtained from CP-EUG and CFP-EUG emulsions at different EUG concentrations are shown in Figure 6.7 and Table 6.3, respectively. At all conditions, CP and CP-EUG films showed significantly higher tensile strengths (17.9 to 51-fold) and Young's modulus than CFP and CFP-EUG films. However, it is important to note that the CFP films were much more flexible, and they showed 2.4 to 4.3-fold higher elongation at break than CP films. It should also be reported that the increase in EUG concentration between 1% (w/w) and 3% (w/w) did not

cause a considerable change in the mechanical properties of both types of films. These results clearly showed the different film making characteristics of CP and CFP.

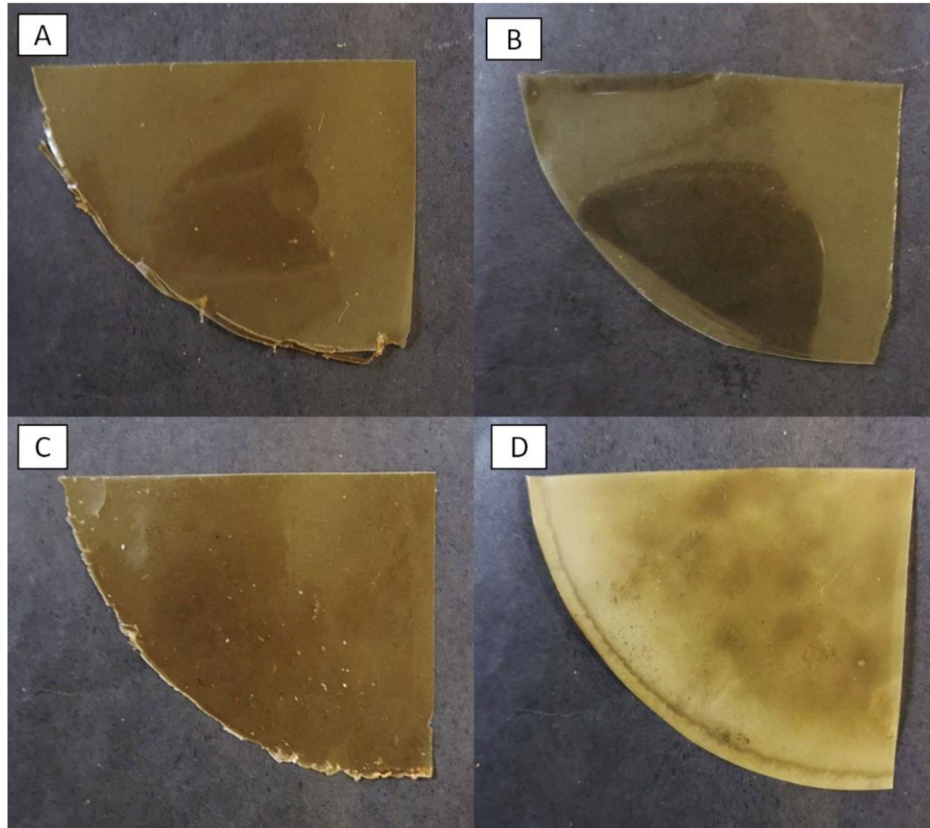

Figure 6.7. Photographic images of pectin films: (A) CFP; (B) CP; (C) CFP- EUG (2%, w/w); (D) CP-EUG (2%, w/w).

6.4.5. Morphology of Pectin Films

SEM micrographs of cross-sectional morphology for CFP and CP films with and without EUG are shown in Figure 6.8. Control films prepared with CFP and CP both had

a dense appearance (Figures 6.8A and 6.8B). However, control CP films exhibited a more uniform morphology than control CFP films. CFP films, in particular, contained some aggregates, possibly arising from insoluble proteins originated from seeds of fig fruit. In contrast, CFP-EUG and CP-EUG films revealed a morphology characterized by an extensive distribution of droplets and void pores (Figure 6.8C and 6.8D). It appeared that part of the EUG remained in the films as emulsion droplets, while some other EUG droplets evaporated to form some voids (pores). Noteworthy, the EUG emulsion droplets were distributed homogenously along with the film matrix, with no signs of accumulation close to the film surface. Nisar et al. (2018) observed similar morphologies in citrus pectin films incorporated with clove bud essential oil (CEO). However, these authors observed coalescence of CEO droplets close to the film surface, which was ascribed to phase separation between pectin and the essential oil, as CEO concentration was increased from 0.5 to 1 or 1.5% (Nisar et al. 2018).

Table 6.3. Mechanical properties of CFP and CP films at different EUG concentrations.

EUG (%, w/w)	Tensile strength (MPa)	Elongation at break (%)	Young's modulus (MPa)	Film thickness (µm)
CFP films				
-	0.53 ± 0.10^{a}	27.44 ± 1.01^{a}	0.03 ± 0.01^{a}	83.7 ± 6.30^{d}
1	0.39 ± 0.06^{a}	24.58 ± 1.93^{ab}	0.024 ± 0.00^{a}	102 ± 4.41^{c}
2	0.22 ± 0.01^{b}	21.43 ± 0.77^{b}	0.012 ± 0.00^{b}	118 ± 6.22^{b}
3	0.24 ± 0.02^{b}	22.15 ± 1.65^{b}	0.012 ± 0.00^{b}	133 ± 0.98^{a}
CP films				
-	9.49 ± 0.93^{B}	6.35 ± 1.64^{A}	3.72 ± 0.48^{BC}	81.1 ± 17.4^{C}
1	14.2 ± 0.86^{A}	8.87 ± 1.35^{A}	4.78 ± 0.05^{A}	102 ± 2.76^{BC}
2	8.55 ± 0.86^{B}	8.87 ± 3.83^{A}	3.01 ± 0.27^{C}	123 ± 5.50^{AB}
3	12.3 ± 0.66^{A}	7.11 ± 0.86^{A}	4.34 ± 0.09^{AB}	147 ± 9.88^{A}

*Data are shown as mean ± standard deviation of triplicate measurement. a-d and A-C at each column denote a statistically significant difference separately for CFP and CP data, respectively ($p \leq 0.05$).

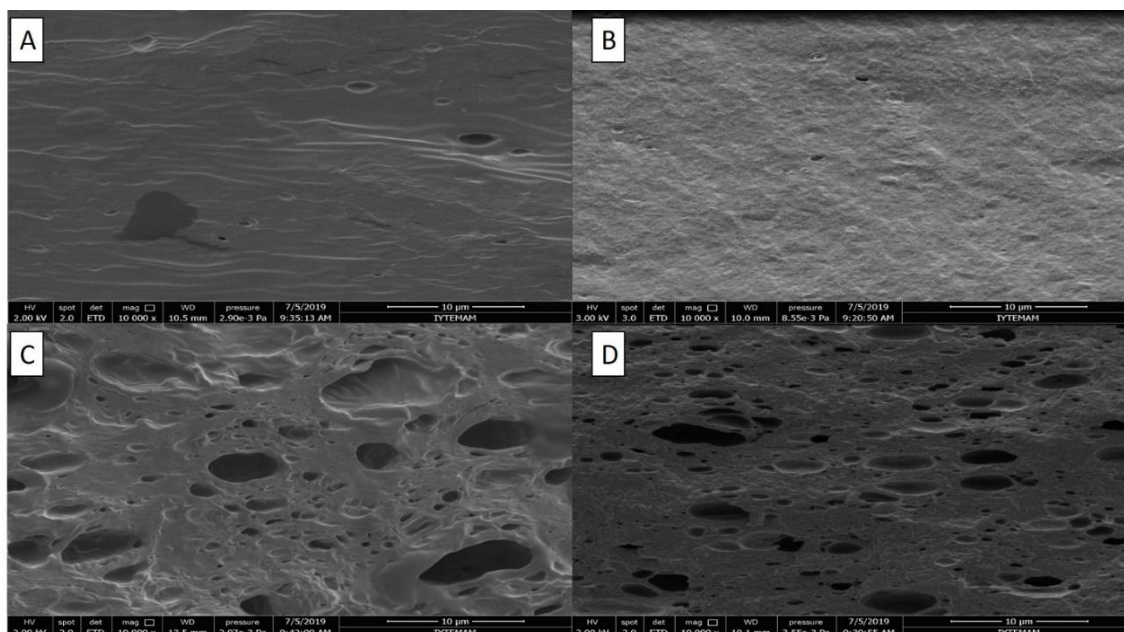

Figure 6.8. Effect of 2% (w/w) EUG on the cross-sectional morphology of pectin films: (A) CFP; (B) CP; (C) CFP-EUG; (D) CP-EUG (Magnification: 10,000×).

6.5. Conclusions

This study clearly showed the good potential of pectin-EUG emulsion-based edible coatings to reduce the risks associated with contaminated *Listeria* on webbed-rind melons. The antimicrobial emulsions developed using pectin extracted from sun-dried fig processing wastes and commercial citrus pectin showed similar antilisterial effectiveness on coated webbed-rind melons. However, fig pectin films differed from citrus pectin films in terms of critical EUG concentrations to achieve antimicrobial properties and different physical and mechanical properties that could be exploited to increase the applicability of the developed films on alternative melon cultivars (or crops) that had variable rind characteristics and physiological properties. This work is an example of the valorization of agro-industrial wastes to obtain alternative food hydrocolloids with different functional and technological characteristics.

CHAPTER 7

SUMMARY AND CONCLUSIONS

Within the scope of this thesis, pectin extraction was performed from various fig wastes as fig puree and stalks and low-grade dried figs. The pectins obtained were characterized in terms of molecular and functional properties. The main results of this thesis are summarized below.

- To produce pectin from sun-dried figs and their wastes such as stalks, the economically feasible hot citric acid extraction method was preferred over ultrasound-assisted extraction due to its acceptable pectin yield and low SLR that enables minimum alcohol use in the purification step. The use of citric acid instead of mineral acids was considered a green approach by the pectin industry.
- The application of ultrasound-assisted extraction in combination with hot acidic extraction provided a slight increase in pectin yield, but the ultrasound-assisted extraction time required to achieve this yield at maximum power was close to that of acidic extraction. Moreover, the ultrasonic extraction needed 3-4 times larger SLR that maximized ethanol use during purification.
- The enzyme-assisted extraction process using cellulase enzyme was applied as an alternative method. This procedure enabled the fig pectin to be extracted under mild conditions without decreasing the degree of esterification, but the efficiency of this process to extract pectin remained significantly lower than those of the other methods.
- Fig stalk separated as waste during the processing of high-quality sun-dried figs and low-grade dried figs were determined as the most suitable materials for pectin extraction. In particular, CSP has some better advantages than fig fruit pectin

originating from its high extraction yield, molecular properties (GA and DE) and low protein content. High degree esterification and galacturonic acid level were easily achieved with PSP by the classical repeated ethanol precipitation method.

- The FT-IR characterization performed for CFP, CSP and PSP showed that these pectins have peaks quite similar to those of commercial citrus and apple pectins. However, the sugar analyses performed by the enzymatic method showed that the fig pectins showed less linearity, but more branching than the citrus pectin. The PSP showed more molecular similarity to citrus pectin than the crude pectins. PSP showed acetylation and esterification levels close to those of commercial pectins. In contrast, CFP had the lowest degree of esterification and the highest degree of acetylation among studied pectins.
- The molecular weight parameters of CSP showed that this pectin had similar Mn and Mw/Mn ratios with citrus pectin, but slightly different Mw value than citrus pectin.
- The protein levels of CSP and PSP were at the level of citrus pectin, but CFP had a significantly higher protein content due to proteins coming from the fig seeds.
- The functional characterization studies showed that PSP and CSP can create emulsions that demonstrate a significantly higher stability compared to CFP and commercial pectins. The PSP also showed a higher viscosity than the crude fig pectins. Therefore, it can be used as an alternative to citrus pectin in emulsion-based foods (e.g., salad dressings, sauces, ice creams, milk- and yogurt-based drinks, etc.), emulsion films and coatings. Additionally, the outstanding water holding capacity of PSP makes it ideal for formulating meat and meat products such as meatballs, burgers, sausages, etc.
- Detailed gelation and texture profile analysis tests conducted under standard gelation conditions for highly esterified pectins showed that fig pectins required higher concentrations than commercial pectins for gel formation. The minimum gel formation concentration for fig pectin was 1.2% for CFP and PSP and 1.75% for CSP. The most distinctive gel properties demonstrated by fig pectins were the formation of harder and more fragile gels compared to commercial pectins. However, the hardness and fracturability of CFP gels were much higher than those of CSP and PSP gels. Additionally, although fig pectin gels had a strong gummy nature, they showed lower internal and surface adhesion than citrus pectin. These

results indicated that fig pectins can be used to produce sweet jelly food products (jelly candies, jam, marmalade) with textural properties quite different from those of commercial pectins.

- The gelation tests conducted under standard gelation conditions for low esterified pectins showed that low esterified fig pectins did not form a gel with $CaCl_2$ in low-sugar environments. This indicated that they cannot form regular carboxyl groups in block-wise manner or create ordered "egg-box model" conformations.
- The pectin-EUG emulsion-based edible coatings showed significant antimicrobial activity against *Listeria innocua* both on agar surface and on contaminated webbed-rind melons as a model food.
- The physicochemical, mechanical and barrier properties of fig pectin films showed that these novel pectins are good candidates as citrus pectin alternative edible packaging and coating materials. The significantly more flexible nature of fig pectin films than citrus pectin films suggested that these novel pectin films could show a better resistance against brittle film formation problem in low humidity environments. In addition, fig pectin films showed better moisture barrier properties than commercial pectin films. Finally, it should be noted that the water vapor barrier properties of fig pectin films could be improved by crosslinking with $CaCl_2$.

The overall results of this thesis clearly showed that the fig stalk containing stalk and a residue of fruit flesh (flesh accounts for 1-1.5% of the total fruit weight) is a very suitable source for pectin extraction. Fig stalk is a waste obtained from the production of pasteurized intermediate moisture high-quality portion-packed figs which is currently being disposed of without being utilized or occasionally used for animal feed. For the first time in the literature, stalk waste has been utilized into value-added crude and purified pectins characterized for their techno-functionality and edible films with details. The outstanding functional properties of purified fig stalk pectin showed that commercial production of this hydrocolloid could provide huge financial benefits to Turkish sun-dried fruit industry. The low-grade dried fig fruits could also be utilized as a source of pectin, but preparations of this pectin suffer from protein and polysaccharide impurities. Therefore, crude fig fruit pectins could be better commercialized as soluble dietary fiber preparations than pectin preparations.

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APPENDICES

APPENDIX A

STANDARD CURVES USED IN THE ANALYSES

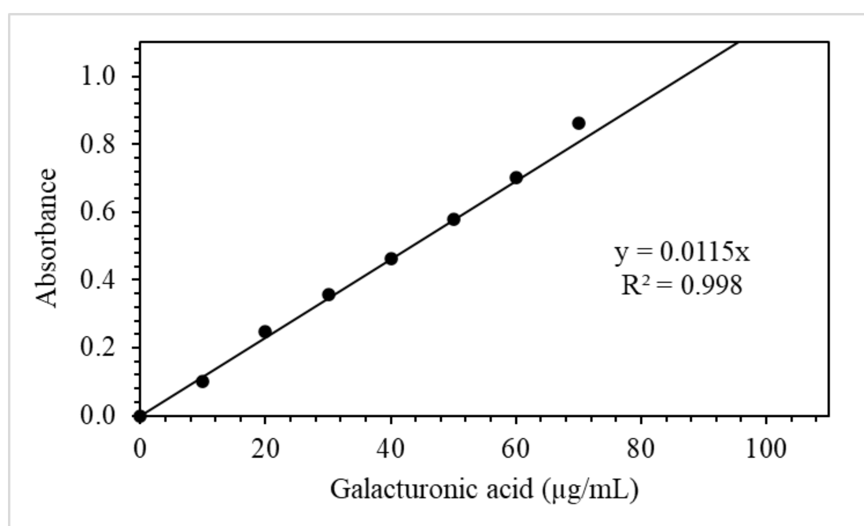

Figure A.1. Galacturonic acid standard curve.

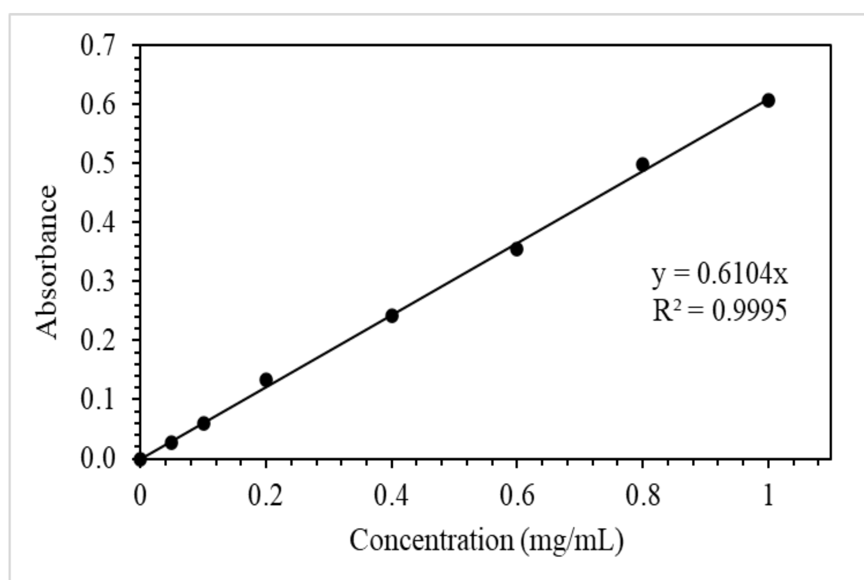

Figure A.2. Bovine serum albumin (BSA) standard curve for soluble protein concentration.

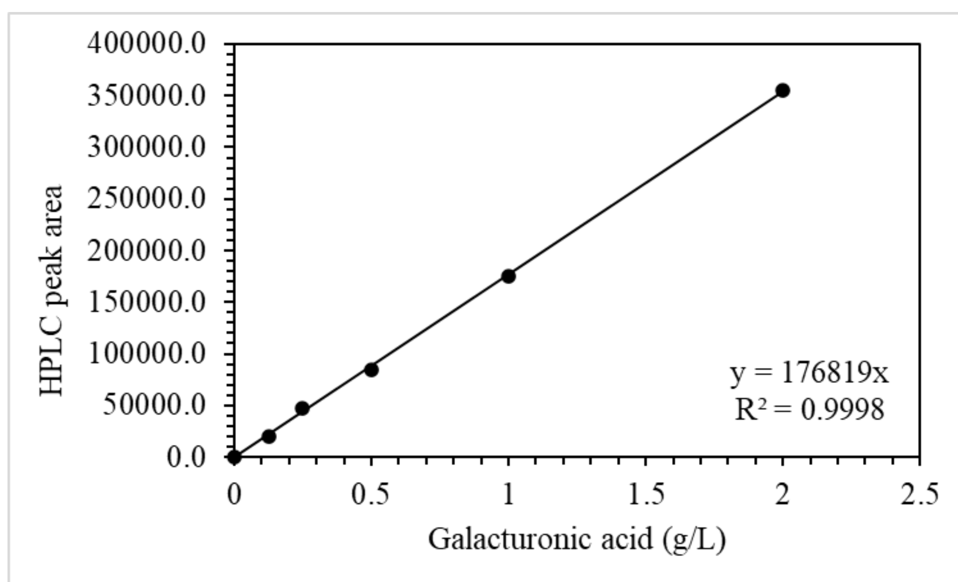

Figure A.3. Galacturonic acid standard curve for HPLC.

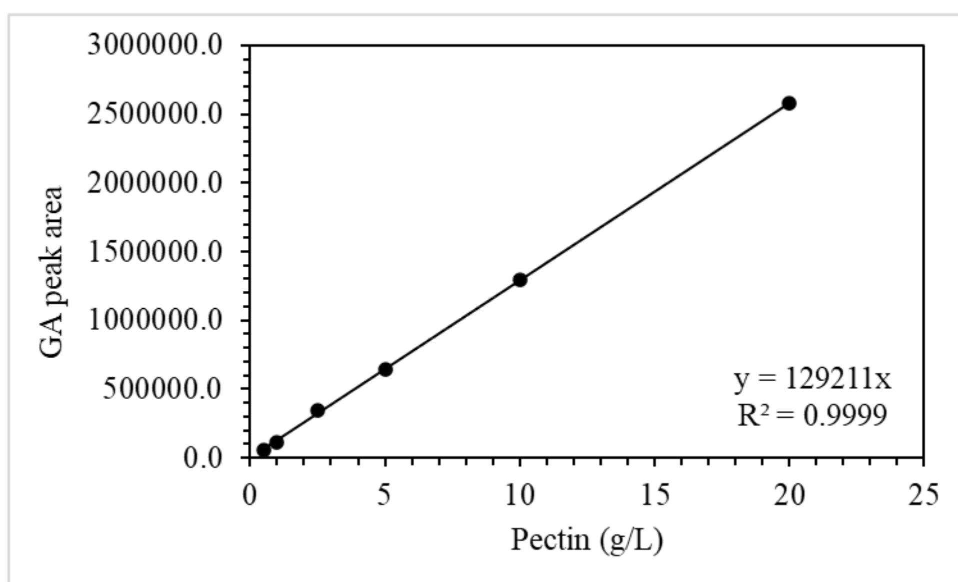

Figure A.4. GA peak area vs pectin content for HPLC.

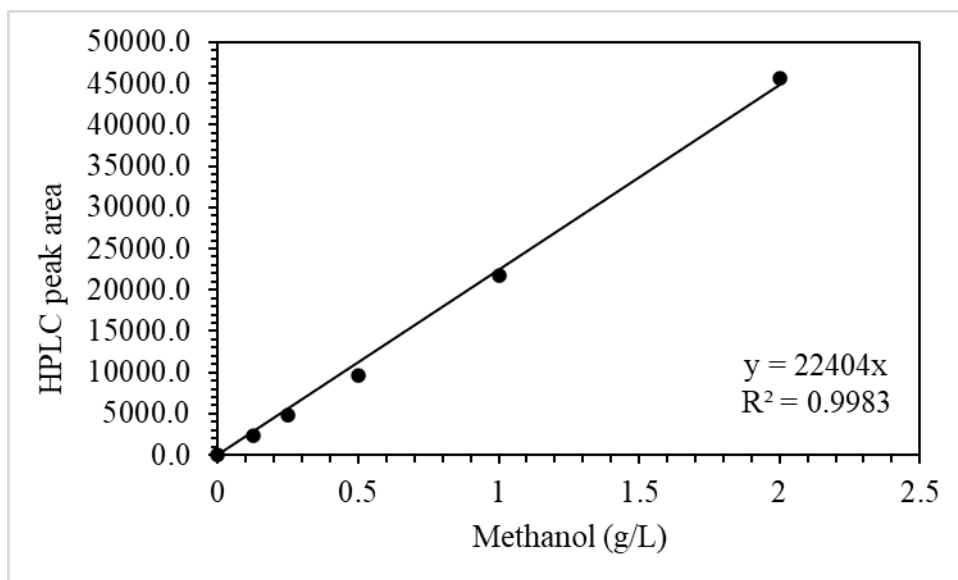

Figure A.5. Methanol standard curve for HPLC.

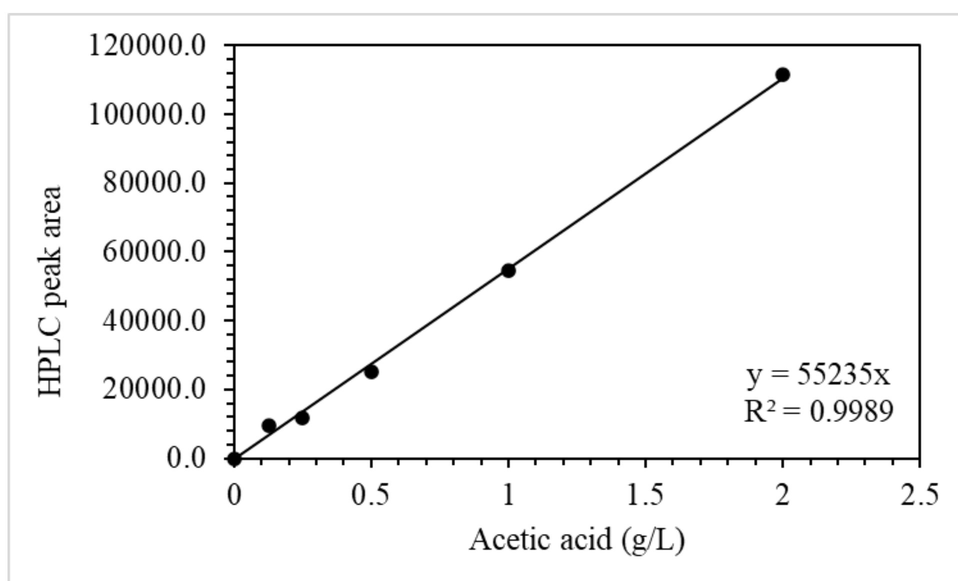

Figure A.6. Acetic acid standard curve for HPLC.

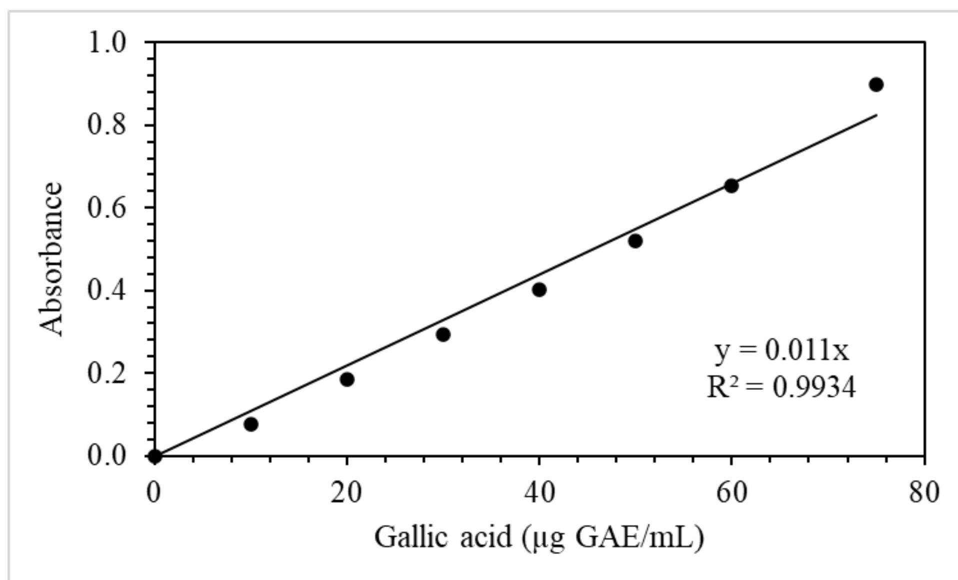

Figure A.7. Gallic acid standard curve for total phenolic content.

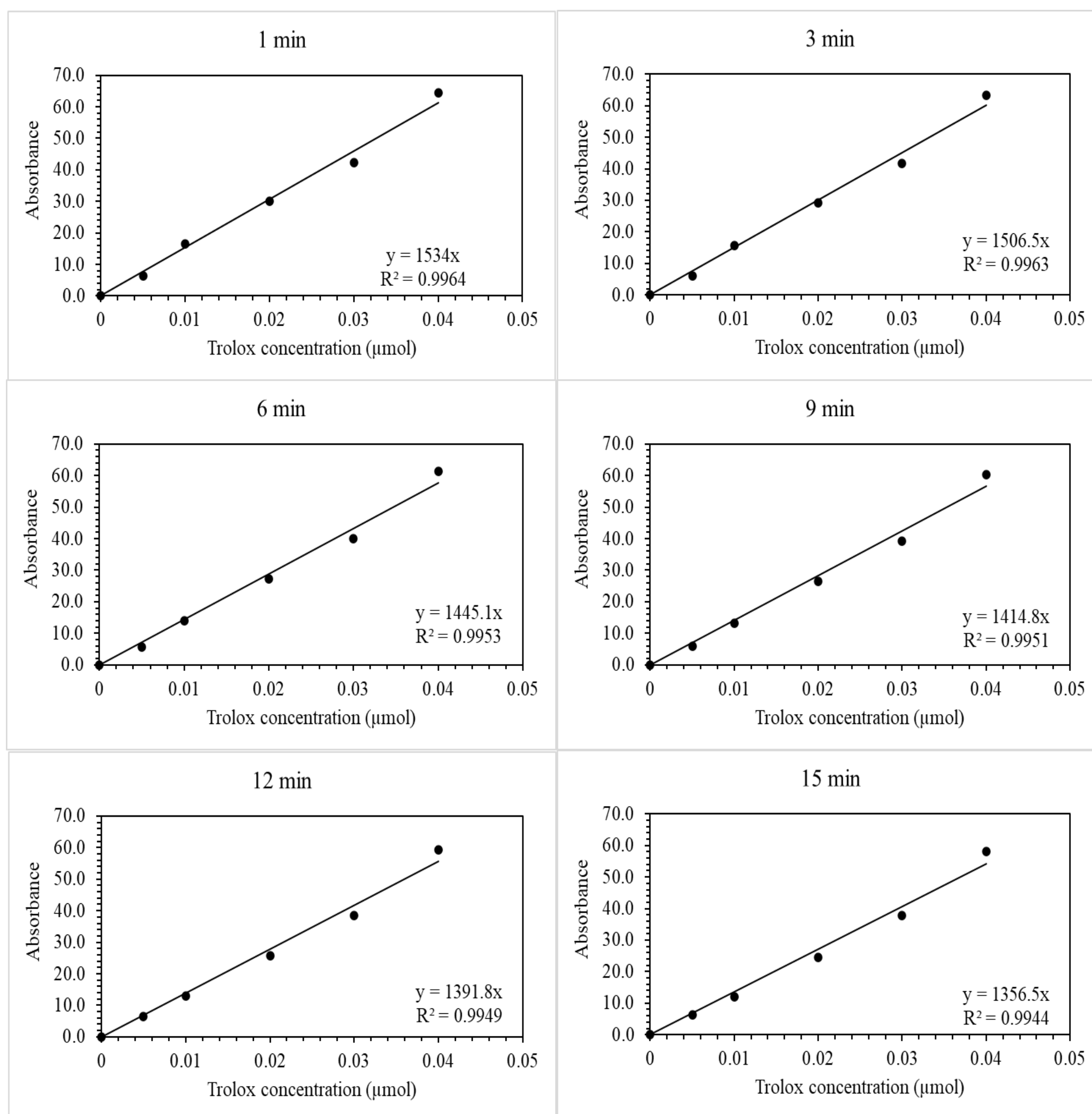

Figure A.8. Trolox standard curve for antioxidant activity based on TEAC.

APPENDIX B

CORRELATION TABLES

Table-B.1. Correlations between mechanical and molecular properties of pristine pectin films.

Sample	Tensile Strength (MPa)[a]	Elongation at break (%)[a]	Young's modulus (MPa)[a]	WVP ($g.mm.m^{-2}.day^{-1}.kPa^{-1}$)[a]	Solubility (%)[a]	Transparency (%)
Protein	N.S.	N.S.	N.S.	N.S.	-0.510, 0.052	-0.757, 0.001
TPC	N.S.	N.S.	N.S.	N.S.	N.S.	-0.899, 0.000
GA	0.802, 0.000	-0.734, 0.002	0.717, 0.003	-0.762, 0.001	0.851, 0.000	N.S.
DE	0.726, 0.002	N.S.	0.517, 0.048	-0.905, 0.035	0.915, 0.030	N.S.
DA	N.S.	N.S.	N.S.	N.S.	N.S.	-0.649, 0.009
D-Glc	-0.943, 0.016	0.923, 0.000	-0.989, 0.001	0.626, 0.013	-0.745, 0.001	N.S.
L-Rha	N.S.	0.588, 0.021	N.S.	N.S.	N.S.	N.S.
D-Gal	N.S.	N.S.	0.616, 0.015	N.S.	-0.538, 0.039	-0.852, 0.000
L-Ara	N.S.	N.S.	N.S.	N.S.	-0.628, 0.012	-0.711, 0.003
R-1	N.S.	N.S.	N.S.	N.S.	0.659, 0.008	0.802, 0.000
R-2	N.S.	0.728, 0.002	N.S.	N.S.	N.S.	N.S.
R-3	N.S.	-0.765, 0.001	N.S.	N.S.	N.S.	N.S.
R-4	N.S.	-0.768, 0.001	N.S.	N.S.	N.S.	N.S.
HG	0.667, 0.007	-0.737, 0.002	0.632, 0.011	-0.679, 0.005	0.876, 0.050	N.S.
RG-I	N.S.	N.S.	N.S.	N.S.	-0.612, 0.015	-0.644, 0.010

[a] Data are shown as "Pearson correlation, P-value". N.S. = not significant ($p \geq 0.05$).

Table-B.2. Correlations between mechanical and molecular properties of Ca^{++} cross-linked pectin films.

Sample	Tensile Strength (MPa)[a]	Elongation at break (%)[a]	Young's modulus (MPa)[a]	WVP ($g.mm.m^{-2}.day^{-1}.kPa^{-1}$)[a]	Solubility (%)	Swelling (%)	Transparency (%)
Protein	N.S.	N.S.	N.S.	N.S.	-0.659, 0.008	N.S.	-0.702, 0.004
TPC	N.S.	N.S.	N.S.	-0.745, 0.001	N.S.	N.S.	-0.909, 0.000
GA	0.847, 0.000	-0.794, 0.000	0.852, 0.000	N.S.	N.S.	N.S.	N.S.
DE	0.628, 0.012	-0.604, 0.017	0.664, 0.007	N.S.	0.783, 0.001	N.S.	N.S.
DA	N.S.	N.S.	N.S.	-0.886, 0.046	N.S.	N.S.	-0.699, 0.004
D-Glc	-0.955, 0.011	0.713, 0.000	-0.965, 0.008	0.677, 0.006	N.S.	-0.871, 0.050	N.S.
L-Rha	N.S.	N.S.	N.S.	N.S.	0.528, 0.043	N.S.	N.S.
D-Gal	N.S.	N.S.	N.S.	-0.914, 0.030	N.S.	N.S.	-0.903, 0.000
L-Ara	N.S.	0.524, 0.045	N.S.	-0.874, 0.050	N.S.	N.S.	-0.766, 0.001
R-1	N.S.	-0.516, 0.049	N.S.	N.S.	N.S.	N.S.	0.799, 0.000
R-2	N.S.	0.727, 0.002	-0.515, 0.050	N.S.	N.S.	N.S.	N.S.
R-3	N.S.	N.S.	N.S.	N.S.	-0.604, 0.017	0.714, 0.003	N.S.
R-4	N.S.	N.S.	N.S.	N.S.	-0.615, 0.015	0.713, 0.003	N.S.
HG	0.760, 0.001	-0.835, 0.000	0.790, 0.000	N.S.	N.S.	N.S.	N.S.
RG-I	N.S.	0.595, 0.017	N.S.	N.S.	N.S.	N.S.	-0.658, 0.008

[a] Data are shown as "Pearson correlation, P-value". N.S. = not significant ($p \geq 0.05$).

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