International Journal of Medical Science and Dental Research

Modification of Pectin-Glycine with Indomethacin to treatment the wound and inflammations

Firyal Mohammed Ali *Hameed Muthanna Ahmed

Al-Mustansiriyah University, College of Science, Department of Chemistry, Baghdad, Iraq

ID 46810532

Abstract: This work included preparation new drug polymer as bio adhesive , which have high viscosity and treatment the wounds andexternal inflammation, because it remains inherent to the position of injury fast time, A new bio adhesivepolymer was prepared bymodification of Pectin structure with Glycine as a spacer substituted with drug such as Indomethacinproduced amide polymer. This design carries controlled delivery of therapeutic agents which could release the entrapped drug over an extended period of time due to its biodegradable, nontoxic and slow digesting nature. The prepared drug polymer wsa characterized by FTIR, ^{1}H-NMR spectroscopes, thermo gravimetric analysis TGA and DSC were studied. intrinsic viscosities and Physical properties of all prepared polymer wsa measured, Biological activity was studied for drug polymer,this new adhesive drug biological polymers were applied on different infected mice and wounds, It gave good results and compliance mice infected with a full recovery by a short period of time.

Keywords: Pectin , Indomethacin,Glycine polymer,

I. INTRODUCTION

Pectin is an important structural polysaccharide with functions in plant growth, morphology and development .[1]Fruit ripening involves Pectin.

breakdown induced by the enzymes Pectinase and Pectinerase leading to cell separation .[2] Pectin today is waste from the juice industry in the form of citrus peel, mainly from lemon and lime. Other commercial Pectin are sourced from orange peel and apple and an emerging new source is from sugar beet from the sugarindustry. Pectin are conceivably the most complicated of the natural plant carbohydrates, both in terms of their chemical composition and their physical chemical structure. [3]homogalacturonans (HG), rhamnogalacturonan. The degree of methylation (DE) refers to the ratio between methylated and non-methylated GalA. Pectin with high DE is known as HM.[4] Pectin also be partially esterified with acetic acid in certain plant species such as sugar beet [5]Again, the ratio between acetylated and non-acetylated GalA is referred to as the degree of acetylation (DAc) [6].well-characterised component constitutes the 'hairy' regions of Pectin or rhamnogalacturonan I (RGI) regions. RGI consists of a backbone composed of a repeating disaccharide of GalA and rhamnose (Rha) residues [-4)-α-D-GalA-(1,2)-α-L-Rha-(1-]n'[7]. Pectins themselvesare ,depending on the source the sidechains may contain minor amounts of other sugars such fucose[8], xylose, mannose[9], glucose [10] glucuronic acid and methyl esterified glucuronic acid and, in some, phenolic[11],extraction with hot acid. The peel or pulp is suspended in 70-90°C water with nitric acid to pH 1-3 for 3-12 hours. This is then filtered and the fluid that has been leached from the plant material is concentrated and mixed with alcohol to precipitate the pectin, after which it can be dried and milled [12],[13]. Extraction may be optimised to preserve or isolate parts of the pectin depending on what is being researched. An alternative method of pectin extraction is microwave-assisted flash extraction. As hot acid extracted pectin undergoes a relatively long period of heating, it experiences thermal degradation, whereas microwave[14-18].

To create pectins for different functions, the pectin has to be modified. This is easily achieved as pectins are unstable and susceptible to changes in pH and temperature. Pectin has good stability in aqueous solution at around pH 3-4. At acidic conditions lower than pH 3 glycosidic bonds and methyl-ester linkages may undergo hydrolysis. The rate of hydrolysis increases with higher temperature and lower pH [19-20]. Hydrolysis of the sensitive neutral sugar side chains may lead to an increase in GalA content and decrease in neutral sugar content. Studies have shown that mild acid hydrolysis causes the progressive release of sugars accompanied by their rapid degradation[21-24].

II. Materials

Pectin was purchased from Fluka and dried at 110°C for about 2 h to remove absorbed moisture. were Glycine and Indomethacin were from Sigma Chemicals , All other solvents and reagents were of analytical grade

III. Instrumentation

Melting point was measured using Thermal Microscope (Kofler-method), and Reichert thermovar, Stuart SMP 30. Infrared spectrophotometer measurements were performed using Shimadzu FT-IR 8400 series Fourier Transform, ^{1}H-NMR spectra were measured with a bruker spectrophotometer model ultra-shield at 300.13 MHz in DMSO-d6.U.V-Visible double beamscanning spectrophotometer VARIAN (UV-Vis)-100 Conc,.

IV. Controlled drug release :-

Release of PGI was studied. 100 mg was added continuously in (100 ml) buffer solution at (37 ^{0}C). The wavelength of λ_{max} was measured at different periods and different pH values (4 –10) by using UV spectrometer. The sample was analyzed by UV- spectroscopes periodically withdrawn the sustained release was measured by the mole fraction constructed from UV.

Determination of median lethal (LD_{50})[25]

In this experiment 6 mice (three male, 3 female) were administered with 3 %PGI. The mice werewatched for 72 hours ,the LD_{50} value revealed that PIG has no toxic effect onmice.(P. Armitage(2005)

Results And Discussion.

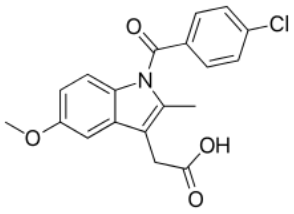
Chemical modification of Pectin graft Glycine (PG)

(3.0 gm, 0.018 mole) of pectin dissolved in (25ml) of Dioxin (3gm, 0.03 mole) of Glycine was added one drop of HCl as a catalys, the mixture was added and heated about (30) minutes at (60 0C), using water bath, the colored product was produced (90%)a softening point was (100-1200C)

Substituted of PG with Carboxylic drug:-

(0.30 gm, 0.0022 mole) of pectin g-Glycine was dispersed in (10 ml) of acetone, (0.7 gm, 0.003 mole) indomethacin dissolved in (5ml) of dioxin, (1 ml) of DMF was added to the mixture, the mixture was refluxed with stirring about 1 hour at (90 ^{0}c), the colored solution was filtered, the filtrate was isolated and the solvent was evaporated, the light brown viscose product PG1 was washed with di ethyl ether two times and dried at (50 ^{0}C) in a vacuum, conversion (90 %). all physical properties were listed in table (1).

PG1 Table **1 Physical properties of prepared polymer**

Pol.	-Drug	Color	Softening point ^{0}C	Conversion ratio %
PG1	 Indomethacin	Black	100-120	80

V. Results And Discussion:

Modification of Pectin-Glycine with Indomethacin:-

Pectin si important structural components of cell walls of the soft, non- woody parts of fruit, vegetables and terrestrial plants. Within a living plant it is an important structural polysaccharide with functions in plant growthA new bio adhesivepolymer was prepared bymodification of Pectin structure with Glycine as a spacer substituted with aonim drug such as Indomethacinproduced amide polymer. ,

Pectin+Glycine-------->- **+---thionyl chloride---------->-**

The prepared drug polymer wsa characterized by FTIR

Figure (1) FTIR spectrum of natural polymer (pectin) showed absorption band at (3250 cm-1) of (O-H) group and (C-O-C) ether absorption bands at (1012-12119 cm-1), band at (2961) cm-1 due to (C-H aliphatic) stretching.

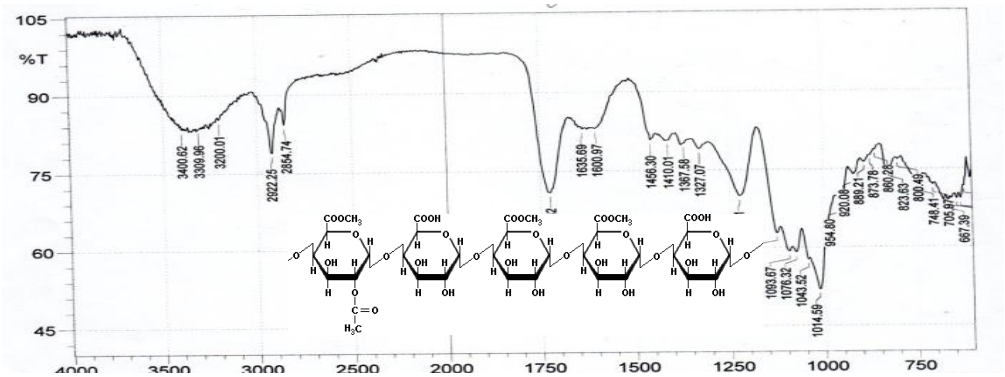


Figure (1) FTIR spectrum of pectin

FTIR spectrum of (PG) pectin-Glycine gave the characteristic absorption of carbonyl group of anhydride band was appeared at (1776 and 1855cm^{-1}) in addition to the pectin backbone absorptions.

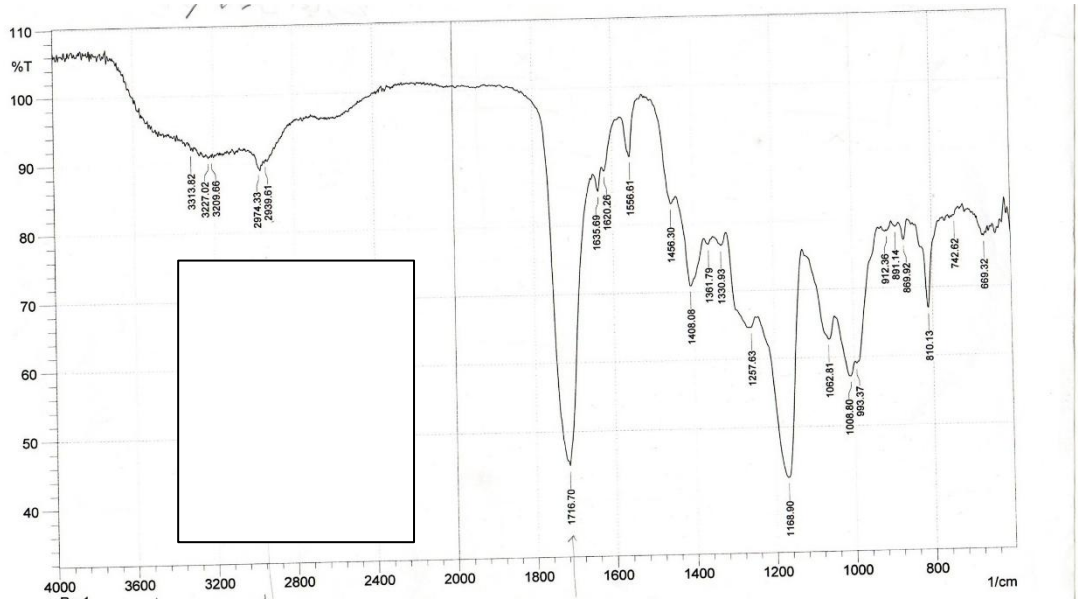
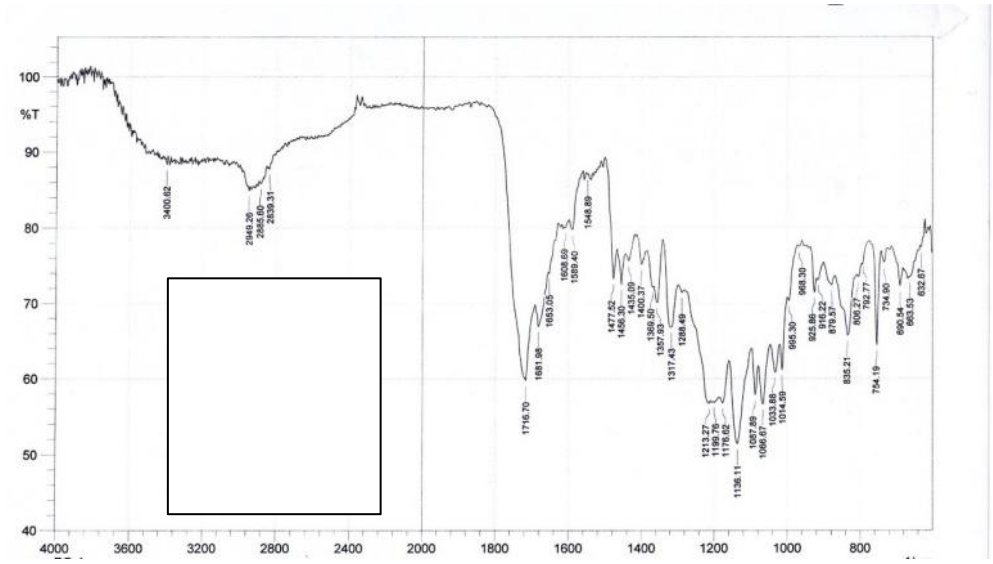


Figure (2).FTIR Spectrum of polymer PG



Figure(3).FTIR Spectrum of Polymer PGI

polymer containing hydroxylic group as characteristic absorption was appeared at (3338 cm^{-1}) in addition (-NH) at (3220 cm^{-1}), absorption of amide (CONH) appeared at (1633 cm^{-1}), band at (1706) cm^{-1} due to (C=O) stretching vibration of acid.

^{1}H-NMR spectrum of prepared polymer was showed in Figure 4 of pectin, which showed the following signals

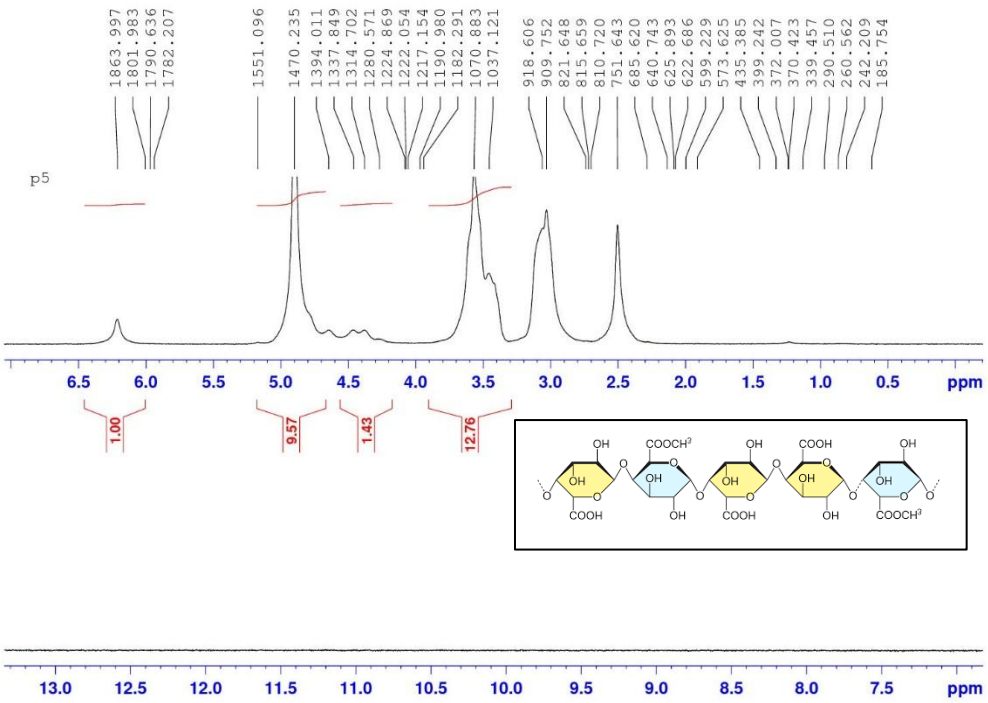
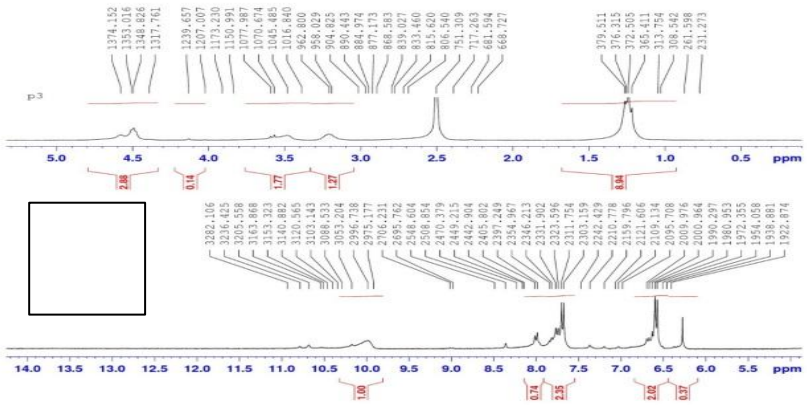


Figure (4) ^{1}H-NMR Spectrum of Pectin

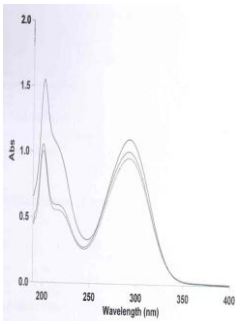
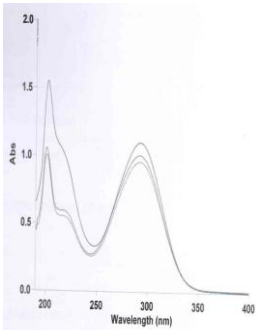
1.25 ppm (Triplet, 3H, CH_3), 2.34 ppm (Triplet, 2H, CH_2), 3.6 ppm (Triplet, 2H, CH_2)



Figure(5)^{1}H-NMR Spectrum of prepared polymer PGI

Figure 5 ^{1}H-NMR Spectrum of prepared polymer PGI showed the following signals.

Figure 5[1]H-NMR Spectrum of GPLshowed1.2 ppm (Triplet, 3H, CH3), 6.2 ppm (Singlet,1H, CO-NH amide), 7.8–7.9 ppm (3H, Aromatic ring), 4.5 ppm (Singlet, OH for pectin), 12.0 ppm (Singlet, 1H, NH). This work included preparation new drug polymer as bio adhesive , which have high viscosity and treatment the wounds and external inflammation, because it remains inherent to the position of injury fast time, This design carries 7 controlled delivery of therapeutic agents which could release the entrapped drug to extended period of time and slow digesting nature.

Figure (6) UV  **Spectrum of PGI**  **hydrolysis of**

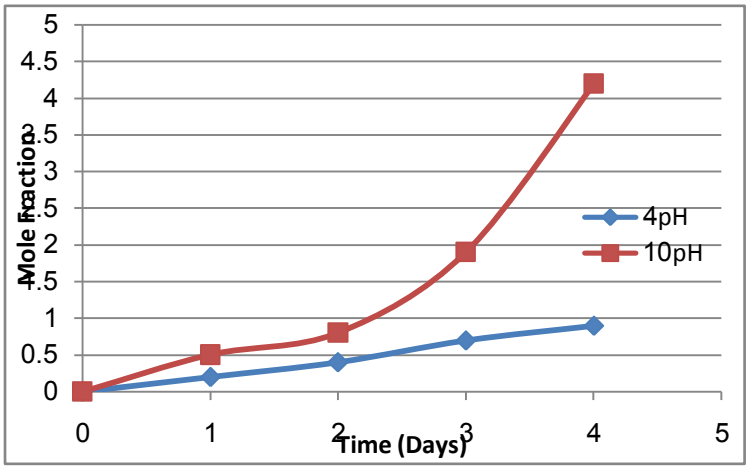


Figure (7) Controlled Release of PGI in pH4 and PH10 at 37 oC in Different pH Values

This new adhesive drug biological polymers were applied on different infected mice and wounds, It gave good results and compliance mice infected with a full recovery by a short period of time.

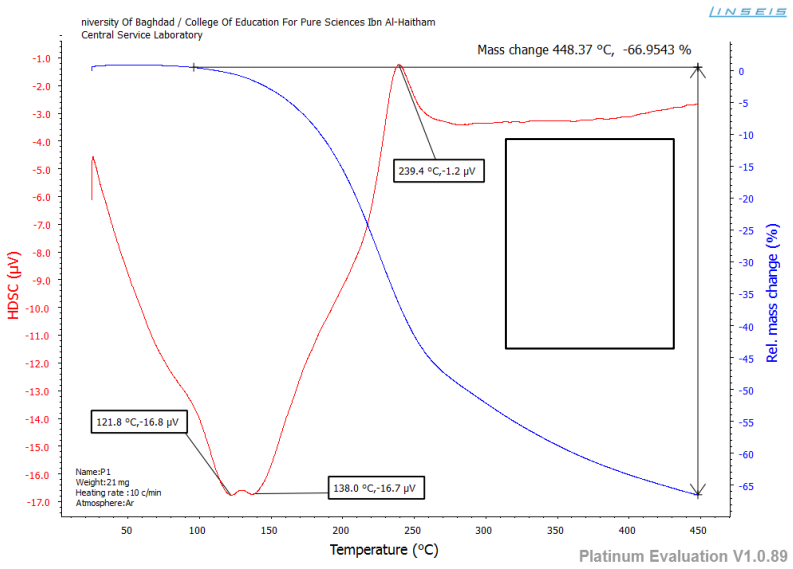


Figure (8)DSC and TGA Analysisof PGI

Thermal stability of prepared saw investigated by (TGA and DSC) TGA indicated the high thermal resistance and showed their steps of weight loss-temperature. This high thermal resistance indicated the high interaction between amide hydrogen bonding through the polymer chains. Several thermal stability parameters were determined from TGA and DSC curves. The change in weight was measured as a function of temperature which gave valuable information about the thermal stability

Weight loss temperature (Ts), which was determined from the TG curve, which represents the temperature at which the sample lost of its total weight In this study (17-22) mg. was taken from the prepared polymers under a programmed heating rate of 10 ^{0}C /mint. under inert atmosphere , (N_2 gas 50 ml/mint). Thus the weight-loss vs. temperature thermo grams were recorded and analyzed. The above parameters which were determined for some of the prepared compounds, were explained and listed in the Table (2).

Table (2a) TGA analysis of some PGI

No. drug polymer	Temperature	Losses weight%
PG	138	43, 38
PGI	239	70.6

Table (2b) DSC analysis of some drug polymers

No. drug Polymer	Onset Temp. ^{0}C	Peak Temp. ^{0}C	ΔH J/g
PG	55.5	59.7	46.61
PGI	138	259	43.61

.

VI. Conclusions:

A new bioadhasivewas prepared bymodification of Pectin structure with Glycine as a spacer, and substitutedwithIndomethacin produced amide polymerPGI. This design carries controlled delivery of therapeutic agents which could release the entrapped drug over an extended period of time due to its

biodegradable, nontoxic and slow digesting nature The new drug polymer was investigated .The prepared drug ePnitc was analyzed in different pH values at 37 ^{0}C*in vitro* study and controlled drug release was compared at zero time and after many days.. It was concluded that modified drug release with extended drug action via slow release and *in vivo* performance was noted to be promising. this new adhesive drug biological polymer saw applied on different infected mice and wounds, It gave doog results and compliance mice infected with a full recovery by a short period of time.

Acknowledgement:-

This work was supported by Al-mustansiriyah University, College of Science, Department of Chemistry, Baghdad.

References:-

[1]. McNeil, M., A.G. Darvill, and P. Albersheim, Structure of plant cell walls X. Rhamnogalacturonan I, a structurally complex pectic polysaccharide in the walls of suspension-cultured sycamore cells. American Society of Plant Biologists, 1980. 66(6): p.1128-1134

[2]. Ridley, B.L., 'Neill M.A. O, and Mohnen D., Pectins: structure, biosynthesis, and oligogalacturonide-related signaling. Phytochemistry, 2001. 57(6): p.929-67.

[3]. Fischer, R.L. and A.B. Bennett, Role of Cell Wall Hydrolases in Fruit Ripening. Annual Review of Plant Physiology and Plant Molecular Biology, 1991. 42: p.675-703.

[4]. Mort, A.J., F. Qiu, and N.O. Maness, Determination of the pattern of methyl esterification in Pectin. Distribution of contiguous nonesterified residues. Carbohydr Res, 1993. 247: p.21-35..

[5]. Talmadge, K.W., et al., The Structure of Plant Cell Walls: I. The Macromolecular Components of the Walls of Suspension-cultured Sycamore Cells with a Detailed Analysis of the Pectic Polysaccharides. Plant Physiol, 1973. 51(1): p.158-73.

[6]. Rolin, C., Commercial Pectin Preparations, in Pectins and

[7]. TheirManipulation, G.B. Seymour and J.P. Knox, Editors. 2002. p.222-239

[8]. Colquhoun, I.J., et al., Identification by nmr spectroscopy of oligosaccharides obtained by treatment of the hairy regions of apple Pectin with rhamnogalacturonase. Carbohydrate Research, 1990. 206(1): p.131–144.

[9]. Yapo, B.M., Rhamnogalacturonan-I: a structurally puzzling and functionally versatile polysaccharide from plant cell walls and mucilages. Polymer Reviews 2011.51(4).

[10]. Schols, H.A., et al., A xylogalacturonan subunit present in the modified hairy regions of apple Pectin. Carbohydrate Research, 1995. 279.

[11]. Morris, V.J., et al., The bioactivity of modified PectinfraGments.Bioactive Carbohydrates and Dietary Fibre, 2013. 1(1): p. 21-37.

[12]. Round, A.N., et al., A new view of Pectin structure revealed by acid hydrolysis and atomic force microscopy. Carbohydr Res, 2010.

[13]. Rolin, C., Commercial Pectin Preparations, in Pectins and Their Manipulation, G.B. Seymour and J.P. Knox, Editors. 2002. p.222-239.

[14]. Fishman, M.L., et al., Global structure of microwave-assisted flash-extracted sugar beet pectin. J Agric Food Chem, 2008. 56(4): p. 1471-8.

[15]. Diaz, J.V., G.E. Anthon, and D.M. Barrett, Nonenzymatic degradation of citrus pectin and pectate during prolonged heating: effects of pH, temperature, and degree of methyl esterification. J Agric Food Chem, 2007. 55(13): p. 5131-6.

[16]. Thibault, J.F., et al., Studies of the length of homogalacturonic regions in pectins by acid hydrolysis. Carbohydrate Research, 1993. 238.

[17]. Renard, C.M.G.C. and J.F. Thibault, Degradation of pectins in alkaline conditions: kinetics of demethylation. Carbohydrate Research, 1996. 286.

[18]. Kiss, J., β-Eliminative degradation of carbohydrates containing uronic acid residues. Advances in Carbohydrate Chemsitry and Biochemistry, 1974. 29.

[19]. Axelos, M.A.V. and M. Branger, The effect of the degree of esterification on the thermal stability and chain conformation of pectins. Food Hydrocolloids, 1993. 7(2).

[20]. Hotchkiss, A.T., et al., Enzymatic modification of pectin to increase its calcium sensitivity while preserving its molecular weight. J. Agric. Food Chem, 2002. 50.

[21]. Buchholt, H.C., et al., Preparation and properties of enzymatically and chemically modified sugar beet pectins. Carbohydr Polym, 2004. 58(2).

[22]. Sengkhamparn, N., et al., Characterisation of cell wall polysaccharides from okra (Abelmoschus esculentus (L.) Moench). Carbohydr Res, 2009. 344(14): p. 1824-32.

[23]. Nagel, M.D., et al., Enzymatically-tailored pectins differentially influence the morphology, adhesion, cell cycle progression and survival of fibroblasts. Biochim Biophys Acta, 2008. 1780(7-8): p. 995-1003.

[24]. Yu, L., et al., Rhamnogalacturonan I domains from ginseng pectin. Carbohydrate Polymers, 2010. 79(4): p. 811-817.

[25]. Mao, F., et al., Anticancer effect of Lycium barbarum polysaccharides on colon cancer cells involves G0/G1 phase arrest. Medical Oncology, 2011. 28(1): p. 121-126.

[26]. P.Armitage(2005)Statistical methods in medical researchin blank well scientific Publication,USA(1971)Claremunt,M,A.Garcia,ConceptionLapez and Jose Elguero,ARKIVOC (vii),91.