PAIR/T.391/99

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## ABSTRACT

**EFFECT OF GAMMA IRRADIATION ON POLYSACCHARIDE MODEL SYSTEMS.** Cell wall polysaccharides which mainly consist of pectic substances, hemicellulose, and cellulose play a major role on the immediate softening of irradiated fruits. Their degradation mechanism can be elucidated by studying degradation products resulting from irradiation of the cell wall or their components. Studies on irradiation of isolated apple pectin, and alginates as the model systems induced degradation were carried out. Pectin and alginates were first purified then both were irradiated either in solid or in solution state. Effect of gamma irradiation at 15-30 kGy on these treated materials was conducted by analyzing their changes by means of chemical, physical, and various chromatographic methods. The results showed that medium and high doses gamma irradiation could reduce the viscocity of pectin and alginates while HPSEC/GPC analysis for all irradiated polysaccharide model systems revealed that the average number of molecular weight showed a decrease by increasing radiation dose. Storage condition in different relative humidities affected significantly the degree of polymerization of pectin and alginates irradiated in solid state. The formation of 4,5-unsaturated uronosyl residues as a product of cleavage of the pectin backbone - via β-elimination was not found in irradiated pectin as measured by thiobarbituric acid (TBA) test. Irradiation did not cause a β-elimination in the ester groups of pectin as confirmed both by titration, and ion exchange chromatography methods.

*Key words : cell wall, gamma irradiation, polysaccharide model systems*

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## ABSTRAK

**PENGARUH IRADIASI GAMMA PADA SISTIM MODEL POLISAKARIDA.** Dinding sel polisakarida terutama mengandung pektin, hemiselulosa dan selulosa memegang peran aktif pada pelunakan seketika dari buah-buahan iradiasi. Mekanisme degradasi dapat dipelajari melalui produk degradasi dari dinding sel yang diiradiasi ataupun komponennya. Studi tentang iradiasi pektin hasil isolasi buah apel, dan alginat telah dilakukan. Pektin dan alginat dimurnikan terlebih dahulu, kemudian diiradiasi baik dalam bentuk padat maupun larutan. Pengaruh iradiasi gamma dengan dosis 15-30 kGy pada bahan yang diberi perlakuan tersebut dianalisis perubahannya dengan menggunakan metode kimia, fisik, dan kromatografi.Hasil yang diperoleh menunjukkan bahwa iradiasi gamma pada dosis sedang dan dosis tinggi dapat menurunkan viskositas pektin dan alginat sedangkan hasil analisis dengan HPSEC/GPC untuk seluruh sistim model polisakarida yang diiradiasi menunjukkan bahwa jumlah rata-rata dari berat molekul menurun dengan meningkatnya dosis radiasi. Perlakuan penyimpanan pada kondisi kelembapan nisbi yang berbeda berpengaruh nyata pada derajat polimerisasi pektin dan alginat yang diiradiasi pada bentuk padat. Pembentukan 4,5-residu uronosil tidak jenuh sebagai produk hasil pemecahan rantai utama pektin melalui proses eliminasi-β akibat radiasi tidak ditemukan melalui pengujian dengan asam tiobarbiturat (TBA). Iradiasi tidak menyebabkan terjadinya eliminasi posisi β-pada grup ester dari pektin yang dikonfirmasi baik dengan metode titrasi maupun kromatografi pertukaran ion.



*Kata kunci : dinding sel, iradiasi gamma, sistim model polisakarida.*


## INTRODUCTION

A study on the effect of irradiation on extracted cell wall material of mango fruit might not enough to provide conclusive results with respect to the underlying degradation mechanism, since it was extreemely difficult to distinguish

between the primary and secondary reactions within the complex plant tissue. Revealing the exact degradation mechanism of irradiated fruits and vegetables showed to be extreemely difficult (1, and 2). Therefore a study of spesific features of the degradation can only effectively be carried out by utilizing less complex systems (3, 4, 5, 6, 7, and 8). Polysaccharides such as pectin, and alginates are considered useful model system polymers to study the degradation of polysaccharides with structural variations by irradiation *in vitro*. Pectin is being an important constituent of plant cell wall was chosen to study the effect of irradiation. Pectins consist of galacturonic acid residues, partially esterified with methanol groups. As comparison, also two anionic polysaccharides were selected, which contrarily to pectin, are built up of different uronic and residues namely mannuronic acid and guluronic acid in varying proportion and segments.

## MATERIALS AND METHODS

*Sample preparation.* A commercial high methoxyl apple pectin (HMP) with a degree of methylation (DM) of 76% was obtained from Obipectin Ltd., Bischofszell, Switzerland. Two commercial samples of alginates were obtained from Kelco International Ltd., San Diego, California : one having a high mannuronic acid/guluronic acid ratio (M/G 2.5), and one other having a low ratio (M/G < 1).

Desalted HMP was prepared by dissolving 1 g of HMP in 200 ml distilled water under stirring. During additional gentle stirring for 10 min of 7.5 g chlorine-free Amberlite IR 45 and 7.5 g Dowex-OH- form 50 W (50-100 mesh) as ion exchanger were added to the solution. The mixture was then poured slowly into a glass column. A bed of mixed ion-exchangers was formed and rinsed with distilled water until a clear solution was obtained. The filtrate was concentrated using a Buchii rotating evaporator, and precipitated in ethanol/water 80% (v/v). The desalted HMP was subjected to repeated washing at room temperature with ethanol/water 80% (v/v) until the filtrate was sugar free. Absence of sugar in the filtrate as detected by the phenol-$H_2SO_4$ test as described by DUBOIS (9). The

alcohol insoluble residue of HMP was finally rinsed with 96% ethanol, then dried by solvent exchange using acetone followed by ether, and drying at room temperature for 3 days. The dried material was pulverized to a fine powder for about 0.7 mm using a Cullatti hammer mill prior to its use. The alginates were transferred into the acid form according to the method in FOOD CHEM. CODEX (10).

Different humidities were set according to LABUZA (11), IGLESIAS and CHERIFE (12) using saturated solutions of two different inorganic salts, e.g., $K_2CO_3$, and NaBr providing relative humidities (RH) of 40 and 60% respectively. The moisture content of equilibrated samples was determined using a Karl Fisher automatic titrator.

*Irradiation treatment.* A Co-60 source was used and the dose rate of irradiation was 2.5 kGy/h in all treatments. The irradiation was conducted at the Institute for Atomic Sciences in Agriculture (ITAL) Wageningen, The Netherlands. Irradiation of all polysaccharides after conditioning at a given RH was performed as powder with doses of 15 and 30 kGy in closed weighing flasks at room temperature in the presence of oxygen. The moisture content of the samples in powder form ranged from 10-13%.

*Viscosity measurement.* Viscosity was measured in a Ubbelohde capillary viscosimeter as described by DEVENTER-SCHRIEMER and PILNIK (13).

*High- Performance Size- Exclusion Chromatography (HPSEC) / GPC analysis.* Estimation of molecular weight was performed on a SP 8800 HPLC (spectra Physics) equipped with three Bio-gel TSK columns (300x7.5mm) in series (60XL, 40XL, and 30XL; Bio-Rad Labs) in combination with TSK guard column (75 x 7.5 mm) and elution at 30°C with 0.4 M acetic acid/Na-acetate buffer (pH 3.0) at 0.8 ml/min (14). The eluate was monitored by a Shodex SE-61 Refractive Index Detector.

*Thiobarbituric acid test.* Formation of unsaturated degradation products of pectic substances as a result of irradiation was qualitatively determined by the periodate thiobarbituric acid test according to **ROMBOUTS** (15).

*Determination on the degree of methylation.* The degree of methylation of HMP (%)in order to confirm the absence of β-elimination in irradiated pectin and the alginic acid content (%) were determined respectively by a titration method (10).

*High-Performance Ion-Exchange Chromatography (HPIEC) analysis.* The elution behaviour of the charge polysaccharides on a anion exchange column was studied as described by **SCHOLS** *et al.* (16).

## STATISTIC CALCULATION

Analysis of variance of the complete randomized design on η viscosity , and degree of polymerization (DP) were calculated statistically according to **STEEL** and **TORRIE** (17). All samples used in the experimental works were conducted in triplicates.

## RESULTS AND DISCUSSION

A first characterization of the polysaccharides including building units, type of substituents, and weight average of molecular weight (avr.Mw) and number average of molecule (avr.Mn) were carried out. The result revealed that avr.Mw and avr.Mn of HMP pectin were 84,000 and 62,000 Dalton (Da.) respectively, while Alginate with M/G 25 having values in avr.Mw was 68,000 Da., and avr. Mn was 43,100 Da.. The avr. Mw and avr. Mn values of Alginate with the ratio of M/G <1 were 69,000 Da. and 52,000 Da. respectively. The effect of irradiation on the viscosity of selected polysaccharides also showed a decrease in their viscosity by increasing radiation dose. The reduction of viscosity might confirm

that irradiation at doses up to 30 kGy induces depolymerization within the glycosidic linkages in the solid samples. However, this phenomena can not be ruled out yet. Other confirmation data on radiation induces depolymerization in the uronic acid backbone via hydrolysis process is needed. Similar result on irradiated strawberries induced degradation of protopectin followed by a reduction of viscosity was reported by **BELLI-DONINI** and **STRONAIUOLO** (18). An interesting study on hydrolysis of highly esterified pectin induced by enzymic activities was also carried out by **PILNIK and ROMBOUTS** (19). The pectin lyase endo-enzymes depolymerizes the molecule at random, causing rapid drop in viscosity, and only glycosidic linkages next to a methyl ester group are split by a β-eliminative mechanism. Other supporting results were demonstrated in irradiated apple pectin as reported by **SJOBERG** (6) and for Na-alginate as reported by **KING** (20). Those results showed a reduction in molecular weight in which corresponds to the decrease in viscosity. **KUME** and **TAKEHISA** (21) reported that irradiation of 3 types of Na-alginates in powder form at a dose of 50 kGy could reduce the viscosity significantly. They concluded that such an irradiation dose can only be applied for sterilization of Na-alginate as absorbant and coagulant in food industries, but it is not applicable for irradiated Na-alginates as medical purposes neither as thickening nor gelling agent.

This phenomena gives an indication that irradiation of pectin with the doses up to 30 kGy did not cause a splitting of glycosidic linkages next to a methyl ester groups by β-elimination mechanism. Since the viscosity of the charge polymers dropped drastically after irradiation, treated samples were also analyzed by HPSEC./GPC and calculated as a degree of polymerization (DP). The result showed that the DP values of pectin and alginates decreased by increasing radiation dose while storage condition at two different relative humidities might affect the DP of each sample significantly. These two atmosphere provide sensitive circumstances for pectin molecules as complex structure which some of the carboxyl groups are esterified with methyl alcohol, some are neutralized with cations, and some are free acids. An intensive study on moisture sorption isotherm of pectin as conducted by **TSAMI** *et al.* (22) revealed that high methylated pectin exhibit different behaviour compared to low methoxyl pectin.

At low relative humidity less than 60% the sorbed moisture of high methylated pectin is very low, and the appearance of the materials is poor. At higher relative humidity, at more than 80%, there is a step increase of the sorbed moisture. At relative humidity in the range of about 60 to 80% the sugars hold the largest portion of water but the pectin molecules have sorbed enough water molecules. In this situation number of interchain bonds of high methoxyl pectin becomes smaller and the remaining carboxyl groups are available for the sorption of water. Loosing pack of biopolymer as indicated by swelling of this substance is due to the intermolecullar distance of methoxyl groups in the chain molecules. Degree of crystallinity also decreases, but availabililty of the polar groups to the water molecules shows an increase. Finally all the swelled polysaccharides goes into a solution in which carboxyl and methoxyl groups are not strong. The dependency of carboxyl and methoxyl groups on the equilibrium moisture content is of importance if a study on degradation mechanism of cell wall polysaccharides as well as pectin induced by irradiation will be elucidated.

Figure 1 shows the elution profile of alginate with the ratio of M/G 2.5 irradiated in powder form during storage.It seems from the figure that irradiated alginate with doses up to 30 kGy showed a shift of the elution profile into longer retention time and the shifting of each top peak after the treatment in comparison to the unirradiated alginate. It is indicated that total degradation has already occured in the irradiated samples before and after storage as direct effect reaction predominant. This phenomena can be approved by the following storage condition. Storage condition at 23°C for 2 months does not influence significantly shifting of elution profile particularly in irradiated samples. The same results also found in irradiated alginate with the ratio of M/G <1. Separate DP calculation using GPC programme revealed that higher in MM block had a DP of about 3-10 while for higher in GG block was about 4-15. It is known elsewhere that some physical properties of irradiated alginates depend on the uronic acid composition and the blockwise arrangement of acid residues.In this study alginate with M/G 2.5 has higher alternating sugar blocks which is more easily degraded than alginate with M/G <1. This finding result is building up a phenomena that the properties of irradiated alginates corresponds to alternate MG blocks to determine the

reduction of degree of methylation was found, and more degradation in pectic substances might occured. Unfortunately, the reduction in the degree of methylation does not occur in enzymic degradation of the pectin molecules induced by glycosidases and lyases activities.

## CONCLUSION

A phenomena can be formulated from the finding results that radiation with doses up to 30 kGy attack cell wall components in the form of model systems in random, and hydrolyzes glycosidic bonds but does not create splitting of high methoxyl pectin backbone through β-elimination reaction due to stability of proton at β-carbon. It seems that irradiation does not easily remove proton in high methoxyl pectin. The proton will be easily removed when the electron density/free electron around the carbon is high.

## ACKNOWLEDGMENT

I wish to thank Prof. Dr. A.G.J. Voragen and Prof. Dr. W. Pilnik for guiding the research work, and staff members of RIKILT Pilot Plant and the Institute for Atomic Sciences in Agriculture (ITAL) for irradiating the samples.

## REFERENCES

1. SIMIC, M.G., "Radiation chemistry of water soluble food components", Preservation of Food by Ionizing Radiation, ed. (JOSEPHSON, E.S., and PETERSON, J.J. eds.) Vol. II, CRC Press, Boca Raton, Florida (1983) 45.

2. IRAWATI, Z., APRIYANTONO,A., VORAGEN,A.G.J. and PILNIK, W., Effect of gamma irradiation on cell wall polysaccharides of mango (*Mangifera indica* L.) var. Tommy Atkins, Submitted to publication.

3. DIEHL, J.F., ADAM, S., DELINCEE, H. and JAKUBICK, V., Radiolysis of carbohydrates and carbohydrate-containing foodstuffs, A. Agric. Food Chem., 26 (1) (1978) 16.

4. RAFFI, J., AGNEL, J.P., BOIZOT, C., THIERY, C. and VINCENT, P., Glucose oligomers as model to elucidate the starch radiolysis mechanism, Die Starke 37 (7) (1985) 228.

5. DRIJVER, L.D., HOLZAPFEL, C.W. and der LINDE van, H. J., High performance liquid chromatographic determination of D-arabinohexos 2 ulose (D-Glucosone) in irradiated sugar solutions : application of the method to irradiated mango, J. Agric. Food Chem., 34 (1986) 758.

6. SJOBERG, A.M., The effect of irradiation on the structure of apple pectin, Food Hydrocolloids 1 (4) (1987) 271.

7. BEYERS, M.,DRIJVER,D., HOLFZAPFEL, C.W., NIEMANDS, J..G.,PRETORIUS, I. and der LINDE van H.J., "Chemical consequences of irradiated of subtropical fruits", Recent Advances in Food Irradiation, ed. (ELIAS, P.S., and COHEN, A.J., eds.), Elsevier, Biomedical Press, The Netherlands (1983) 171.

8. PURWANTO, Z.I., BROEK, van de, L.A.M., SCHOLS, H.A., PILNIK, W. and VORAGEN, A.G.J., Degradation of low molecular weight fragments of pectin and alginates by gamma irradiation, Acta Alimentaria (27) (1998) 29.

9. DUBOIS,M., GILLES,K.A., HAMILTON, J.K., REBERS, P.A. and SMITH, F., Colorimetric method for determination of sugars and related substances, Anal. Chem., 28 (1958) 350.

10. FOOD CHEMICAL CODEX, Purity test on galacturonic acid, degree of amidation and degree of esterification, FCC III/Monograph, 3rd edition, National Academic Press, Washington, D.C. (1981) 77 and 215.

11. LABUZA, T.P., Moisture Sorption Practical Aspects of Isotherm Measurement and Use, The American Association Of Cereal Chemist, USA. (1984).

12. IGLESIAS, H.A. and CHIRIFE, J., Water Sorption Parameters for Food and Food Components, Hand Book of Food Isotherms, Academic Press, New York (1982).

13. DEVENTER-SCHRIEMER, van, W.H. and PILNIK, W., Studies on pectin degradation, Acta Alimentaria (16 ) (1987) 143.

14. ROMBOUTS, F.M., Occurences and properties of bacterial pectate lyases, PhD Thesis, Wageningen Agricultural University, Wageningen, The Netherlands (1972).

15. SCHOLS, H.A., POSTHUMUS, M.A. and VORAGEN, A.G.J., Structural features of hairy regions of pectins isolated from apple juice produced by the liquefaction process, Carbohydrate Research (206) (1990) 117.

16. SCHOLS, H.A., REITSMA,J.C.E., VORAGEN, A.G.J. and PILNIK, W., High performance ion exchange chromatography of pectins, Food Hydrocolloids (3) (1989) 115.

17. STEEL, R.G.D. and TORRIE, J.H., Principle and Procedures of Statistic. A Biometrical Approach, Second Edition, Mc. Graw Hill International Editions, Statistic series, Singapore (1981).

18. BELLI-DONINI, M.L. and STRONAIUOLO, M.R., Pectin changes in the ripening of irradiated and stored strawberries, J. of Food Science (34) (1969) 509.

19. PILNIK, W. and ROMBOUTS, F.M., "Pectic enzymes", Enzymes and Food Processing ed. (BIRCH, G.G., BLAKEBROUGH, N. and PARKER, K.J. (eds.) Applied Science Publisher Ltd., London (1981) 105.

20. KING, K., Changes in the functional properties and molecular weight of Na-alginate following irradiation, Food Hydrocolloids (2) (1994) 83.

21. KUME, T. and TAKEHISA, M., Effect of gamma irradiation on sodium alginate and carragenan powder, Agric. Biol. Chem., (47) (1983) 889.

22. TSAMI, E., VAGENAS, G.K. and MARINOS-KOURIS, D., Moisture sorption isotherms of pectins, J. of Food Processing and Preservation (16) (1992) 151.

23. HEYRAUD,A. and LEONARD, C., Structural characterization of alginates by liquid chromatography, Food Hydrocolloids (4) (1990) 59.

24. CLARKE, I.D., "Effect of ionizing radiation on the storage properties of fruits", Preservation of Fruit and Vegetables by Irradiation Especially the Tropics (Proc. of Panel by Joint FAO/IAEA Division of Atomic Energy in Food and Agriculture) Vienna, 1-5 August 1966 (1968) 65.

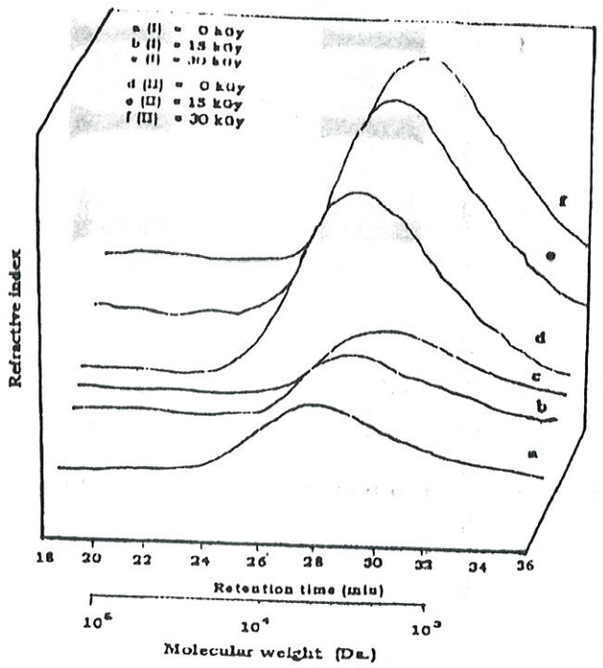


Figure 1. Elution profiles of irradiation aginate M/G 2.5 before (I) and after (II) 2 months storage at 23°C as detected by HPSEC

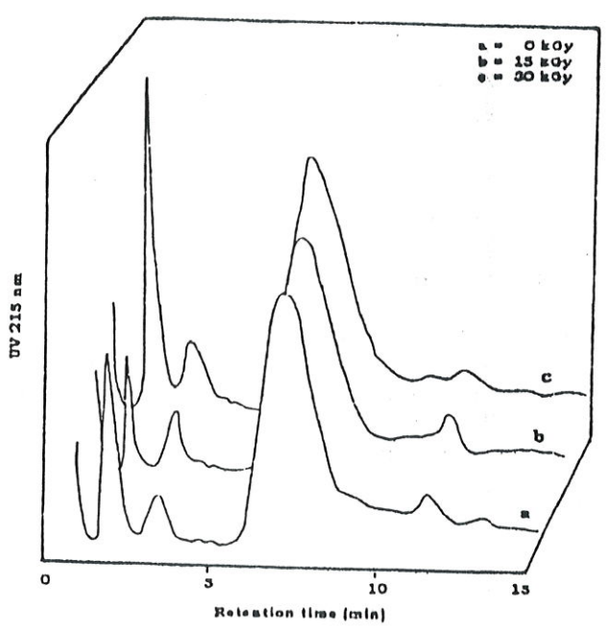


Figure 2. Elution profiles of irradiated high methoxyl pectin as detected by HPIEC