EFFECT OF GAMMA IRRADIATION ON CELL WALL POLYSACCHARIDE MODEL SYSTEMS

By

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ABSTRACT

EFFECT OF GAMMA IRRADIATION ON CELL WALL POLYSACCHARIDE MODEL SYSTEMS. (H.M. Aman Wirakartakusumah as chairman, A.G.J. Voragen, Soewarno T. Soekarto, Dedi Fardiaz, and Anton Apriyantono as members of committee).

Irradiation is an alternative preservation method which can be utilized to extend the shelf life of agricultural products by eliminating number of insects, and decreasing microbial growth effectively. Irradiation at low dose can inhibit some physiological and biochemical changes of certain fruits, resulting in a delay of the ripening stage, and of their senescence. However, irradiation of fresh fruit at pasteurization dose might create an adverse effect on the fruit texture by losing their tissue integrity.

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 cell wall or from cell wall components.
Cell wall polysaccharides were isolated from mango flesh by ethanol insolubilization prior to low dose radiation exposure. A sequential extraction using Na₂CyDTA was applied to the irradiated materials followed by further isolation steps to obtain pectin, hemicellulose, and cellulose fractions. These fractions were then analyzed to determine their neutral sugars, uronic acid and cellulose contents, elution behaviour of the charge polysaccharides, and molecular weight distribution.

The result revealed that ionizing radiation at a dose of 10 kGy subjected to isolated cell wall polysaccharides could degrade the macromolecules into smaller components. Some of the glycosidic bonds were split by irradiation as resulted by increasing some solubilized of sugar components and the reduced of molecular weight. However, such information was insufficient to obtain an explicit data on degradation mechanism of irradiated mango fruit due to the complexity of the substrates. Therefore, it is advantageous to carry out experiments with polysaccharides model systems to elucidate specific feature of degradation by irradiation.

Studies on irradiation of isolated apple pectins, alginates and arabinans as the model systems induced degradation were carried
out. Pectins and alginates were first purified then both irradiated either in solid or in solution state. Effect of gamma irradiation at 15-30 kGy on these treated materials was conducted by analyzing some changes using chemical, physical, and different chromatography methods.

The results show that medium and high doses gamma irradiation could reduce the viscosity of pectin and alginates, while irradiation did not cause β-elimination in the ester groups of pectin as confirmed by titration and ion exchange chromatography methods. 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. HPSEC/GPC analysis for all irradiated polysaccharides model systems revealed that the average number of molecular weight showed a decrease by increasing radiation dose. Storage condition in 2 different relative humidities affected significantly the degree of polymerization of pectin and alginates irradiated in solid state.

A more detailed insight on the degradation mechanism of irradiated polysaccharides can be seen clearly by irradiating the
lower molecular weight fragments prepared from the same model systems.

Polygalacturonic acid (PGA) consists of a galacturonan backbone with free methoxyl groups, was fragmented into lower molecular weight components via an enzymic degradation. PGA is the best substrate for endopolygalacturonase which specifically hydrolyze the glycosidic linkages next to free carboxyl groups.

Low molecular weight fragments of PGA obtained by enzymic degradation, as well as low molecular weight fragments of pectin (methyl esterified polygalacturonic acid), and alginates obtained by mechanolysis have been used to study the mechanism of radiation-induced degradation.

By means of high-performance anion-exchange chromatography (HPAEC), the degradation of specific entities induced by irradiation and the formation of new compounds were measured. The low molecular weight fragments of PGA, pectin, and alginates show an increasing reduction in the degree of polymerization upon exposure to increasing irradiation doses. An alginate with high ratio of mannuronic to guluronic acid appeared to be more sensitive towards irradiation than an alginate having a low ratio. Degradation of polygalacturonic acid fragments by
irradiation can be ascribed to hydrolytic cleavage of glycosidic bonds, although HPAEC analysis revealed that other unknown products were also released during the irradiation process. Storage at -20°C of various irradiated PGA fractions did not affect significantly their average molecular weight and had no effect on their HPAEC elution pattern. A phenomena can be postulated from the whole results that gamma irradiation of cell wall polysaccharides both in solid and solute states are likely more degradation than cross linking. The mechanism seems to be a hydrolysis in random by splitting glycosidic bonds, but does not create neither β-elimination reaction nor new double bonds on high methoxyl pectin. Irradiation induced degradation of the oligomers in solute state could produce some new fragments, and considered as intermediate radiolytic products.

*Key words*: alginates, cell wall, irradiation, mango, oligomers, pectin.
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By

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To complete a requirement for a Doctor degree in the field of food science

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Zubaidah Irawati was born on August 15, 1950 in Pekalongan, Central Java. She spent her childhood with her parents, in Semarang until she graduated from senior high school in 1969. She went to Bogor Agricultural University to pursue her study on the faculty of technology and mechanization on agriculture and graduated in 1977.

Since 1979 she has been working for food preservation group at radiation processing division National Atomic Energy Agency in Jakarta. From 1981 to 1982 she participated a International Facility for Food Irradiation Technology (IFFIT) fellowships sponsored by IAEA for 6 months in Wageningen, The Netherlands. Prior to her PhD programme from September 1986 to July 1988 she studied on the degradation of cell wall polysachharides of tropical fruits using various commercial enzymes at the Department of Food Science/Food chemistry and Microbiology Division, Wageningen Agricultural University, Wageningen the Netherlands sponsored by Netherlands Government as a grant scholarship. In September 1988
she officially admitted as a PhD student from the same university. Starting from September 1989 - August 1992 she worked for her PhD research on cell wall polysaccharides as substrates to elucidate their degradation mechanism induced by gamma irradiation using various chromatography methods. She transferred her credit points to Bogor Agricultural University under food science post graduate programme on February 1996 to finish her study. She is married, and having 2 children.
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LIST OF ABBREVIATIONS

A. AIS : Alcohol insoluble solids
ALKSP : Alkali soluble pectin
AM : Amylomaize
Ar: Arabinose
AUA : Anhydroy uronic acid
Avr. Mn : number average of molecular weight
Avr.Mw : weight average molecular weight
B. B : Bread wheat
C. CELL : Cellulose
CL : Chemi Luminescence
Co-60 : Cobalt-60
Cs-137 : Cesium-137
CyDTAIR : Cyclo hexane diamino acetic acid insoluble residue
D. Da. : Dalton
DHA : Dihydroxy acetone
DM : Degree of methylation
D max/D min = U : Radiation dose uniformity
DP : Degree of polymerization
E. ESR : Electron Spin Resonance
G. G : Glyceraldehyde
Gal : Galactose
GC/MS : Gas chromatography/Mass spectra
L-GG block : L-guluronic-guluronic acid block
Gluc : Glucose
GPC : Gel performance chromatography
H. H : Haricot bean
H2M : 2-hydroxy malonaldehyde
HBA : Highly branched arabinan
HEMI : Hemicellulose
HMP : High methoxyyl pectin
HMw : High molecular weight
HPAEC : High performance anion exchange chromatography
HPIEC : High performance ion exchange chromatography
HPLC : High performance liquid chromatography
HPSEC : High performance size exclusion chromatography
K. kGy : kilogram
L. LL : Lyoluminescence
LMw : Low molecular weight
Mw : molecular weight
M : Manioc
MAC : Modified atmosphere coating
Man : Mannose
MeV : Million electron volt
MG-Block : Mannuronic-guluronic block
M/G 2.5 : Mannuronic-guluronic acid ratio 2.5
M/G <1 : Mannuronic-guluronic acid ratio <1
MM-Block : Mannuronic-mannuronic block
MMw : Medium molecular weight
MN : Maize
MNP : 2-methyl-2-nitrosopropane
Mrad : Megarad
N : Na2CyDTA : Na cyclohexane diamino tetra0 acetic acid
Na2CyDTASP : Na-cyclohexane tetra acetic acid soluble pectin
NIR : Near infra red
NMR : Nuclear magnetic resonance
NS : Non-suspended
P : Potato
PAD : Pulse amperometric detector
PED : Pulse electrochemical detector
PG : Polygalacturonase
PGA : Poly-galacturonic acid
PME : Pectin methyl esterase
Prop-Gly Al : Propylene glycol alginate
R : Rice
Rh : Rhamnose
S : Suspended
\eta Spec. : Specific viscosity
T : TBA : Thiobarbituric acid
TL : Thermoluminescence
U : USD : US dollar
UV : Ultra violet
W : WM : Waxy maize
X : Xy : Xylose
I. INTRODUCTION

Tropical fruits such as banana, papaya, pineapple, and mango are important commercial fruits which serve not only as table fruits, but also as raw materials in several fruit processing industries for producing among others nectar, juice, jam, and fruit flakes.

Fruits production in Indonesia increased from 4.53 million tons in 1989 to 5.62 million tons in 1993. It was also reported that productivity of tropical fruits increased from 7.53 ton/ha in 1988 to 12.23 ton/ha in 1993 (Winarno, 1995). Mango is one of the exotic tropical fruits which could also be promoted as non-oil commodities to increase country devisa. Export value of mango from Indonesia to various importing countries in the world showed an increase from 579 thousand USD in 1990 to 857 thousand USD in 1992 (Purwadaria et al., 1995).

It has been well understood that changes in fruit texture, flavour and appearance after harvesting become very important quality standard criteria for evaluation of fresh fruits in the market. Fruits are commonly harvested at a mature stage after
which softening takes place inside the fruit and continues even after harvesting if the conditions are suitable.

Textural changes induced by tissue softening of fruit is a result of the break down of cell wall polymer network either due to biochemical changes or actions of hydrolytic endogenous enzymes which convert the insoluble constituents into soluble and edible ones (Biale, 1960; Pilnik and Voragen, 1970; Hulme, 1970; Pilnik and Voragen, 1984, and Pilnik, 1984) but less intensive studies on irradiated cell wall polysaccharides induce depolymerization (Diehl et al., 1978; and Kon, 1978). Lavan and Ali (1993) reported that an extensive softening in mango appears to be related to more extensive modification of the wall pectins.

Most fruits contain large amount of water, as well as solutes which could be readily utilized by microorganisms, insects, and other pests. This situation makes the fruits prone to spoilage, specially under storage condition which prevail in most tropical countries. Furthermore, such economically important tropical fruits normally have a limited postharvest life of about 2 weeks.

There are some technological approaches which might only be effective in improving the shelf-life of particular fruits, i.e.,
storage in a modified atmosphere (MA) /control atmosphere storage (CAS), or in a modified atmosphere packaging (MAP) (Purwadaria, 1998), and coating (MAC) treatments, storage at low temperature, hot water dipping, and dipping in calcium salt as reported by Jasim et al. (1968). Ionizing radiation using gamma rays, X-rays and high-energy electron beams can also be used for such purposes (Satin, 1993).

Gamma irradiation as a preservation technique is widely used to extend the storage life of some fruits and vegetables for different purposes such as sprout inhibition of bulbs and tubers (Matsuyama and Umeda, 1983), delaying post harvest ripening and senescence of fruits (Akamine and Moy, 1983), insect disinfestation for quarantine purposes (Tilton and Burditt, Jr., 1983), and reducing microbial loads by single treatment of low irradiation dose or in combination with hot-water dipping (Moy, 1983).

Purwanto and Maha (1986) reported that gamma irradiation induced tissue softening in fresh mango was due to the solubilization of protopectin into soluble pectin. Since insoluble pectic materials maintain cellular adhesion in plant tissues, the destruction of protopectin by ionizing radiation appears to be the
cause of radiation induced softening of fresh fruits which is mostly considered as an adverse effect. Alteration of protopectin in the intercellular areas allows the tissue cells to separate resulting in loosing of texture.

Adverse physico-chemical changes occurring in irradiated fresh fruits and vegetables using high radiation doses are surface and flesh darkening as a result of enzymatic browning, and immediate softening due to the degradation of long chain polysaccharides (Murray, 1990; Clarke, 1961; Yasia et al., 1987; and Sjoberg, 1987). The extent of changes in different structures and chemical constituents of irradiated fruits and vegetables depends on several factors such as radiosensitivity of each individual product, temperature of the environment and the absorbed irradiation dose through the materials (Somogyi and Romany, 1964).

The ionizing gamma radiation degrades at least the major polymers in the primary cell wall of plant materials (pectic substances, hemicelluloses and cellulose), (Clarke, 1968; and Maxie and Sommer, 1968) although the mechanism has not been clearly explained (Mc. Ardie and Nehemias, 1956; Rouse and
Denisson, 1968; Belli-Donini and Stronaiuolo, 1969; Echandi et al., 1970; and Diehl et al., 1978).

The degradation of the cell-wall polysaccharides such as in pectin induced by gamma irradiation is due to hydrolysis by random attack of the glycosidic linkages within the polymers leading to smaller units having the same general structure (Kertesz et al., 1956; McArdie et al., 1956; Maxie and Sommer, 1968; Skinner and Kertesz, 1960; and Clarke et al., 1961) caused by free radicals generated by irradiation (Murray, 1990). Likewise in the immediate softening process is not attributable to accelerate enzymatic hydrolysis of the wall components (Maxie and Sommer, 1968). Irradiation of pectic substances derived from apple probably resulting in random hydrolytic cleavage of the galacturonan backbone yielding fragments of lower molecular weight (Sjoberg, 1987).

Fresh fruits such as mango is normally exposed to irradiation as constituents coexisting major and minor components of complex fruit systems. However, it is extremely difficult to elucidate the degradation mechanism occurs in irradiated mango fruit induced softening in situ. Pattern and composition of the
radiolytic products and degree of sensitivity of these polysaccharides are strongly depended on the particular environments, e.g., type of cell wall, physical orientation of the polysaccharides molecules, and other artifacts.

It is estimated that some changes observed in an irradiated isolated cell wall polysaccharides as individual model systems might also give similar pattern when the irradiated complex fruit systems containing such polysaccharides is observed.

Different speculation on the study of degradation mechanism of irradiated fruits and vegetables proposed that free radical species are probably responsible for pectin depolymerization (Ko, 1978; and Urbain, 1986), and the limit degradation of pectins caused by radiation was explained by competitive OH· scavengers present in apples (Sjoberg, 1987).

Due to the complexity in structure of mango fruit, it is difficult to obtain an explicit data on the degradation mechanism of the irradiated fruit induced softening. The studies were aimed to obtain some information on degradation products of irradiation of isolated cell wall polysaccharides in mango fruit, and
irradiated polysaccharides model systems. The effect of radiation on these various polysaccharides is estimated by direct and indirect effect reactions through different sample preparations in order to elucidate their degradation mechanism.

HYPOTHESIS

Immediate softening in gamma irradiated mango fruit is mainly due to hydrolysis process occur in the primary cell wall polysaccharides via a specific pattern through preferable attacked of free radicals to the cell wall components, resulting new fragments with lower molecular weight.
II. LITERATURE REVIEW

A. Irradiation of Fresh Fruits and Vegetables

Ionizing radiation applied as a cold preservation method seems to be an alternative for fruits and vegetables in order to reduce their losses and to improve their quality. Sample conditions (degree of ripening, water content, uniformity in size, etc.), and radiation parameters (dose, presence and absence of oxygen, temperature, etc.) are of important factors in the irradiation of fruits and vegetables.

The chemical mechanisms of depolymerization reaction in irradiated polysaccharides can be investigated in two ways, i.e., by evaluating the structure of the radiation induced-radicals through interpretation of different physical methods such as Electron Spin/Paramagnetic Resonance (ESR) spectra, and by an exact structure analysis of the radiolysis products.

Irradiation may induce severe depolymerization of macromolecules resulting in structure loss which must be considered as an adverse effect. Various studies on the effect
of gamma irradiation on physico-chemical changes of fruits and vegetables have been reported by many investigators.

Studies on irradiated fresh mango both using single irradiation or hot water dipping, and combination treatment between irradiation at 0.75 kGy and hot water dipping for 5 min. at 55°C revealed that the combination treatment was the best in prolonging the shelf-life of fresh mango for 2 weeks at 25°C and then another 1 week at room temperature (29-30°C) to allow the fruits into normal ripening without affecting some chemical characteristics, and the organoleptic quality (Purwanto and Maha, 1987). Other study has revealed that irradiated mango with doses up to 0.75 kGy did not influence significantly to the amount of glucose, sucrose, fructose and total sugars but after storage the irradiated mango showed more changes of those values than in the control (Singh, 1990). It is also reported that immediately after irradiation the water soluble pectin value of fresh mango was as high as in other fruits but during storage its rate of increase was lower in the untreated samples. A contrary result was obtained in insoluble pectin fraction after storage. The irradiated fraction
decreased but the rate of decrease was lower than in the unirradiated mango.

Study on radiolytic changes occurring in mango revealed that irradiation of carbohydrate component produces carbonyl compounds. Using models of carbohydrate derived from mango through different preparations, i.e., solution, juice, pulp and whole fresh mango, the finding result showed that the individual component of the irradiated food was mutually protected. Other experimental work on sugar solution used as model system indicated that radiolytic products of D-arabino-hexose-2 ulose (D-glucosone) was present, but this type of radiolytic products was not found in the irradiated fresh mango (Delincee, 1989).

Physico-chemical analyses of irradiated frozen de-seeded kiwi pulp during storage at -18 °C has been investigated (Lodge et al., 1985). The result revealed that soluble solid fraction, pH and titratable acidity did not change significantly during storage up to 7 weeks and even up to 6 months freezing storage the total pectin remained constant at values of 0.5-0.6%. Howard and Buescher (1989) concluded that softening in cucumber pickles caused by irradiation was primarily
associated with the changes in pectic substances and some changes of galactose in the cell-wall.

Somogyi and Romany (1964) reported that irradiated pears and peaches at 18.3°C with the doses ranging from 3.6 up to 9 kGy gave a clear indication on irradiation induced softening of the fruit tissue. Protopectin was significantly reduced immediately after gamma irradiation, then increased after 4 day storage in comparison to the untreated samples. Pectin value increased twice of that in the control but pectate only showed a slight increase. After storage unirradiated pears were softer than the irradiated ones at moderate dose, but at a higher radiation dose more tissue damage was observed than in the irradiated samples. Softening of pears and peaches were less in irradiation of samples under N₂ atmosphere. Other result was obtained from irradiated pectin of 1% concentration in solute state either under N₂ or oxygen atmosphere. Under those two atmospheric conditions during irradiation, reducing power and viscosity were decreasing immediately. They postulated that an increase in methylesterase activity may also contribute to the initial pectin degradation in irradiated fruits. Similar investigator also studied on the behavior of
irradiated of fresh apple. It is reported that it would not be possible to differentiate between unirradiated and irradiated samples on the basis of changes in molecular weight of pectin. The degree of esterification, however, was decreased by irradiation treatment at low doses. Furthermore they concluded that the depolymerization in the molecular chains could give an effect on the viscosity of apple pectin, and the activity of pectinmethylsterase observed in fruits increased directly after irradiation but the activity tended to decrease after 4 days of storage.

Degradation of cellulose embedded in the apple fruit cell-wall by irradiation would probably contribute to a loss of texture but it seems unlikely that this compound is the primary structural component being degraded (Poa et al., 1980). In irradiated apple the threshold dose for degradation of pectin and cellulose was similar to softening of the tissue (Clarke, 1968).

Immediate increased in water soluble pectin was found in irradiated fresh apple but the pectic substances in irradiated samples had similarity to those control after storage (Clarke, 1968). Sjoberg (1987) reported that pectin content and
molecular weight of fresh apple were not significantly influenced by irradiation with doses up to 10 kGy.

Clarke (1961) mentioned that texture is not an important factor in the quality justification of irradiated fresh apple with the dose up to 2 kGy and under storage condition at 30°C. It is, however, during storage total pectic substances in the fresh apple decreased significantly in both unirradiated and irradiated samples. The same result was obtained in the irradiated pectin fractions at the same dose.

A study on irradiated carrot has been reported by Echandi et al. (1970). Irradiation with the dose of 10 kGy did not alter total extractable pectin nor on total calcium content. Other result on the irradiated carrot as conducted by Ismail et al. (1977) revealed that water soluble fraction and anhydrouronic acid content showed an increased upon irradiation while ammonium oxalate fraction was both increase after irradiation and during storage, but decreased in HCl extractable fraction.

Reduction on the degree of esterification was due to the activation of endogenous pectin methyl-esterase (PME) in irradiated refrigerated cucumber pickles and the pectin
solubilization was the most notable response to irradiation (Howard and Buescher, 1989). Irradiated pickles at 1 kGy showed a reduction of alkali and non-extractable pectin but a marked increase in water soluble and chelating agent soluble pectin.

The activities of PG, PME, and Cellulase in irradiated mature green tomato were demonstrated (Yasna et al., 1987). Using a viscosity measurement seemed that the activities of PG and PME were not influenced directly after radiation up to 5 kGy. After 3 days storage PE activity showed an increase at the dose of 0.5 kGy, as occurred in unirradiated sample, but the activity of PG in the irradiated samples higher than 1 kGy was suppressed during storage. Cellulase activity showed a significant increase directly after irradiation but it was not affected by storage time. Result on irradiated tomato revealed that a reduction in the ratio of proteopectin to total pectin, and in the polyuronides extracted from all fractions, were indicated but hexamethaphosphate soluble pectin showed an increase while water soluble pectin remained constant.

Other authors reported that in tomatoes and potatoes pectic substances are responsible for the changes in total
polysaccharides due to irradiation or storage time before irradiation (Foa et al. and Jona, 1980).

B. Solubility of Primary Cell Wall Induced Softening in Fresh Fruits

Primary cell wall polysaccharides is the major constituent which is responsible for the integrity of plant tissue due to their function as cellular adhesion.

1. Solubility of cell wall polysaccharides induced by enzymic action

The three major components of cell-wall material in fruits and vegetables are pectic substances, hemicellulose and cellulose. The pectin as a complex cell wall polysaccharides member has a backbone which consists of 2 regions, i.e., "smooth" regions which mainly contain of α-D-1,4-galacturonan, and ramified regions introduced as interrupted side-chains which are attached to α-L-(1→2)-linked rhamnose residues present in the backbone interrupting the galacturonosyl residues (Schols, 1995).
Pectic substances is also responsible for the integrity and cohesiveness of plant tissues (Davidek et al., 1990). Solubilization of pectic substances related to the softening process in some tropical fruits such as bananas, mango, orange and apple has been studied by Nwanekezie et al. (1994). The result revealed that in comparison to the other selected tropical fruits mango showed the highest pectin (1.8%) and methoxyl contents and the purity of anhydrogalacturonic acid content of 81.6%.

Softening process in mature fruits is due to the breakdown of the cell wall structure in the fruit pulp by the activities of endogenous enzymes resulting in conversion of insoluble wall-bound protopectin of high molecular weight into water soluble pectin. From many studies it has become clear that pectin is not a homopolysaccharides but that neutral sugars are part of the pectin molecules (Vries et al., 1982).

Pectic substances as non-starch polysaccharides and its endogenous enzymes play the main important role in the process of softening and in their contribution to the metabolic pool of the cell (Filnik and Voragen in Hulme,
1970; and Pilnik and Rombouts, 1981). Polygalacturonase (PG), the enzyme responsible for degrading the (1→4) -
linked galacturonic acid residues, and β-galactosidase are cell
wall hydrolases who plays an important role in tropical fruit
softening. β-galactosidase may function as galactanase and
may act as a pectin debranching enzyme (Lazan and Ali,
1993). Other researchers also reported that in addition to
pectinases the activities of other enzymes such as cellulases
and hemicellulases in the cell wall might contribute to the
softening process of fresh fruits. Further study on the
reducing viscosity caused by enzymatic degradation was
observed by Sreenath et al. (1987). The study showed that a
commercial enzyme, Ultrazym 100, was the most effective
enzyme in reducing the viscosity of mango pulp.

2. Biochemical changes related to fruit softening and
ripening

Softening, increasing sweetness, aroma, and colour
changes are among the most striking phenomena related to
ripening of fruits. The changes might include respiration
rate, metabolism pathways, and individual constituents.
Polysaccharides as important carbohydrate constituents of many foodstuffs, are complex macromolecules as condensation polymer of high molecular weight composing from single monosaccharide units. One unit is connected to the adjacent monosaccharide unit by a glycosidic linkage, such as at C-1 and C-4 position, as oxygen bridge by the elimination of water between the hemiacetal hydroxyl group of one unit and available hydroxyl group of another (Aspinall, 1983; Pilnik, 1984; Belitz and Grosch, 1987; and Davidek et al., 1990).

Fleshy fruits, mostly its edible portion, composes mainly of parenchyme cells and large intercellular air spaces (King Jr. and Bolin, 1989). Parenchyme cells in the pulp mainly contain primary cell walls defined as non-starch polysaccharides. The polysaccharides consisting predominantly by pectic substances which responsible for tissue softening, hemicelluloses, and cellulose microfibrils. The latter are firmly fixed in a matrix of pectic substances and hemicellulosic polysaccharides (Aspinall, 1983; and John and Dey, 1986). It is obvious that textural changes induced softening of the fleshy fruits during ripening is
essentially caused by the conversion of high molecular weight insoluble wall-bound protopectin into water soluble pectin (Hulme, 1970; Plišnik and Rombouts, 1981; Davidek et al., 1990; and Murray, 1990).

Biochemical changes in some fruits such as apricot and mango at different ripening stage have been carried out by Sharaf et al. (1989). It was reported that the percentage and total soluble carbohydrates increased gradually by increasing extent of ripening. At in their immature stage, glucose and sucrose content were highest in apricot and mango, respectively. In ripe and over ripe stages, fructose was the predominant sugar in apricot and whilst in mango is glucose. The minimum value of glucose and xylose were reached at over ripe stage in apricot. In mango fructose tended to increase while glucose and sucrose decreased significantly.

The Philippine mango, Carabao variety, showed some changes in both physical and chemical characteristics during ripening. The most significant chemical changes observed at ripening stage indicated a decrease in starch content but increased significantly in total soluble solids. At
harvest time the pectin content was 0.37-0.53 mg percent and it increased significantly in the ripening stage about twice higher than that in unripe stage (Morga et al., 1979).

Other study on the physico chemical changes of kinnow mandarine orange and pineapple juice concentrates during storage in different temperature reported by Sandhu et al. (1985). They found that pectin content decreased during storage and the losses increase with increasing temperature in those samples.

NIR spectrophotometry in certain circumstances can be applied optimally to estimate total pectic substances, water soluble and insoluble fractions, and methoxyl content of alcohol insoluble solid prepared from fresh peach, apricot, and apple with a better result in comparison to the use of chemical analysis as carried out by Polisello et al. (1990).

It was also reported by Simpson et al. (1984) that a quantitative determination on pectin content of some tropical fruits using Ca-pectate method gave more representative data than that of methanol precipitation.
Methoxyl content of fruits mostly increases with increasing content of pectin.

Saputra et al. (1995) reported that mango (*Mangifera indica* var. gedong) can be classified in to their taste based on sucrose and malic acid concentration as determined by the Near Infra Red Diffuse Reflectance at the wavelength of 1400-1975 nm and calibrated by HPLC.

C. Cell Wall Polysaccharides, and Their Similar Sugars

Building Blocks

1. Pectic Substances

Pectic substances are complex colloidal polysaccharides consisting of α-D-1,4-linked galacturonic acid residues, in which the carboxylic acid groups are partially esterified by methyl groups and partially or completely neutralized by bases (Pilnik and Voragen in Hulme, 1970). Degradation of pectic substances may yield rhamnogalacturonans (slightly and highly branched/ rhamnogalacturonan I and II).
homogalacturonan, arab-inans, galactans and arabinogalactans (Aspinall, 1983; Pilnik, 1984; and John and Dey, 1986). Pectic substances may include protopectin, pectinic acid, pectinates, pectins, pectates and pectic acid (Pilnik, 1970; and Davidek et al., 1990).

Pectins are subdivided according to their degree of esterification and designation of the percent of carboxyl groups esterified with methanol. Pectin with DE > 50% are high methoxyl pectins and pectins with DE < 50% are classified as low methoxyl pectins. Molecular weight distribution of commercial high methoxyl pectins has been analyzed by Brigand et al. (1990) and many other investigators using size exclusion chromatography coupled with low angle laser light scattering methods. It is found that the pectins have molecular weight in the range of 150,000-280,000 Dalton (Da). The araban and galactan chains are present as free polysaccharides found in the lower molecular weight region. It seems that degree of esterification remains constant all along the molecular weight distribution. Intermolecular
distribution of the degree of esterification is narrow but neutral sugars and acetyl content were less stable. Pectins can be extracted by a variety of mild extractants such as alkali, hot water, ammonium oxalate solution, weak acid, and chelating agent (Vries et al., 1981; Shalom et al., 1982; Bartley and Knee, 1982; Baig et al., 1982; Simpson et al., 1984; Renard et al., 1990; Braudo et al., 1992; and Schois, 1995). Pectins can also be hydrolyzed into smaller fragments. The resulting different types of saccharides depend upon the attacked point of the molecular chain by various degradative enzymes such as pectinases, pectin methylesterase, and pectin lyase (Pilnik and Rombouts, 1981; John and Dey, 1986; and Sreenath et al., 1987).

Pectate is particularly found in the middle lamella between adjacent cells. The native pectin plays an important role in the consistency of fruits and vegetables and in the textural changes during ripening, storage, cooking or radiation.
2. Hemicellulose

Hemicelluloses occur covalently linked to the pectic material but non-covalently bonded to cellulose fibrils. They are not extractable in cold water, but extractable in alkali solution.

Hemicellulose in the primary cell wall comprises of three major groups of polysaccharides, i.e., xylans which is a major component in hemicellulose fractions, consisting of backbone polymers of β-1,4-linked D-xylopyranose chains. Hemicelluloses have a net negative charge due to the presence of α-D glucuronic acid residues at the side chain (Fry, 1988); mannan-glucomannans as derivatives of β-1,4-linked manno-pyranose and β-1,4-linked glucopyranose units with various portions of glucose and mannose; and the galactans-arabogalactans, highly branched derivatives of 1,3-and 1,6-linked D-galactopyranose polymers with arabinose side chains (Aspinall, 1983; Pilnik, 1984; and John and Dey, 1986). Galactans, arabinogalactans,
arabinans, xylans and glucomannans are important representatives of hemicellulose.

3. Cellulose

Cellulose is composed of long chains of \( \beta-1, 4 \)-linked D-glucopyranose residues which are held together in fibrils by hydrogen bonds. Aggregates of these fibrils are the basis of the rigid structure of plant cell wall and it may associates with other polymers such as pectin, hemicellulose, lignin and protein (Stevens, 1975).

An organized aggregates of cellulose molecules in plant cell wall is referred to as microfibrils. These microfibrils are composed of crystalline and amorphous regions.

Cellulose is a linear polymer, consisting of D-glucose in \( \beta-1, 4 \) linkage (Aspinall, 1983; Beldman, 1986; and Davidek et al., 1990). Cellulose can be hydrolyzed by strong acid yielding only D-glucose. Partial hydrolysis, however, produces the reducing disaccharide cellulobiose (Voragen et al., 1983; and Murray, 1990). The individual chains of glucan are held together in fibrils by hydrogen bonds between the hydroxyl groups at C-6 and the
glycosidic oxygens of the adjacent chains. A methylation process followed by hydrolysis steps of cellulose yields only 2,3,6-tri-O-methylglucose without branch point. Cellulose is insoluble in water and it is not related structurally to hemicellulose (Doner, 1985; and Beldman, 1986).

The degradation of cellulose is catalyzed by cellulases acting as 8-1,4-glucanglucanohydrolase (Beldman, 1986) resulting in cell-disintegration of pericarp in the ripening stage of fruits but these enzymes do not directly contribute to the ripening (John and Dey, 1986).

4. Alginites

As individual polysaccharides alginites occur in all brown algae as a skeletal component of their cell walls. Algae are extracted with alkalies and then precipitated from the extract by acid or Ca-salts. Alginites are linear copolymers consisting of the following structural units: Mannuronic acid-Mannuronic acid; Guluronic acid-Guluronic acid; and Mannuronic acid-Guluronic acid.
Alginates designated as alginic acid, a polymer similar to cellulose (Stevens, 1975) is a heteropolysaccharides composed of varying and alternating sequences building blocks of \(\beta\)-D-mannuronic acid and \(\alpha\)-L guluronic acids, joined by the \(1\rightarrow4\) linkages.

The \(1\rightarrow4\) glycosidic linkages between \(\alpha\)-L-guluronic acid molecules leads to a diaxial orientation similar to that in galacturonan and equally suitable for an egg box type of chain association with ca-ions (Pilnik, 1984). The ratio of the two sugars (mannuronic/guluronic acids) is generally 1.5 with some deviation with source. The gelling properties of alginate depending upon the amount of guluronic acid in the main chain. Partial hydrolysis of alginate produces some fragments which consist predominantly of mannnuronic or guluronic acid, and also fragments where the two uronic acid residues alternate in 1:1 ratio.

Distribution of uronate residues in alginate chains is related to alginate gelling properties. This statement was approved after using an experimental model of
enrichment of β-D-mannuronic acid and depletion of α-L-guluronic acid in solution fraction as conducted by Garncarek and Garncarek (1993); and Stokke et al. (1993). Their report is basically concerned about the binding strength between low methoxyl pectin and alginate mixed gel.

The differences between low methoxyl pectin and alginate appear on their heat stability under various pH conditions. Under low pH and neutral conditions alginates are more heat stable. Oats and Ledward (1990) have studied the effect of heat on alginates. They revealed that samples with high level of mannuronic acid residue were far less stable against heat.

Alginates are water soluble in the form of alkali, magnesium, amonia or amine salts. The viscosity of alginate solution is influenced by molecular weight and the counter ion of the salt. In the absence of di and trivalent cation or in the presence of a chelating agent, the viscosity is low. However, with a rise in multivalent cation levels, e.g., calcium, there is a parallel rise in viscosity. Freezing and thawing of Na-alginate solution
containing Ca++ ions can increase their viscosity. The viscosity is unaffected within a pH range of 4.5-10. It rises at a pH below 4.5, reaching a maximum at pH 3-3.5 (Belitz and Grosch, 1987).

5. Propylene glycol alginate

Propylene glycol alginate is derivatisation of alginate through a reaction of mannuronic acid with propylene oxide via hydrogen abstraction at C-6 of mannuronic acid (Plinik and Voragen, 1984). It is soluble down to pH 2 and, in the presence of Ca++ ions it will form soft, elastic, less brittle and syneresis-free gels (Gray and Philip, 1991).

6. Arabinan

Arabinans as a member of dietary fibres play an important role in fruits processing. Arabinans are also found abundant in the biomass of crops as waste products. This type of homopolysaccharides presents as pentosan sugars, mainly composed of α-L-arabinofuranosyl residues. They are generally arranged in (1→4) linked chains with
varying numbers of residues substituted with other α-L-arabinofuranosides at the O-2 and/or O-3 position (Beldman et al., 1991; Pilnik and Voragen, 1984).

Arabinans are present in the plant tissues associated with pectic substances as homoglycans such as arabinoxylans, arabinogalactans and arabinogalactan-proteins. It can be isolated by preferential extraction with boiling ethanol or with lime-water in which associated pectic acid will be transformed into insoluble Ca-salt.

Highly branched arabinan can be synthesized by the isolation of 2,3,5-tri, 2,3-di, and 2-O-methyl-L-arabinose on hydrolysis of the methylated arabinans. This type of polysaccharides have some difficulties in the isolation process from other sugar residues, particularly if the arabinans attach to the pectin molecules. Since pectin is unstable against alkaline reagent, the purity of arabinan obtained from such isolation is doubtful. Other method in isolation of arabinan under non degradative condition has still to be developed. Arabinan degrading enzymes become an essential method to elucidate structural characteristics of arabinan (Beldman et al., 1991). However, in sugar beet
polysaccharides the presence of arabinans linkages in association with pectic substances can still be isolated of a linear 1→3 linked arabinan using an α-L-arabinofuranosidase (Aspinall, 1983). In the concentrated apple juice haze become the major problem due to the effect of high amounts of arabinan side chains. A side-branched araban, water soluble araban, links at 1,2 and 1,3 position of araban straight chains with branched points at C₁ and C₅ as glycosidic linkages. An endo 1,5-arabanase is able to break down the side chains of arabinan resulting in a clear solution in the concentrated apple juice (Filnik and Voragen, 1984; and Voragen et al., 1987).

**D. Gamma Irradiation**

Ionizing (γ) radiation, is found in the range of wave length between 10⁻¹⁴ and 10⁻¹⁰ cm, as non-particle energy radiation which causes direct and indirect ionization or excitation of a permanent gas (O'Donnell and Sangster, 1970). It has the highest emission energy level among others and has different ability in penetrating materials compare to α rays and β rays.
As a cold process, gamma irradiation seems to be a promising technique to substitute or to improve the existing preservation methods. This technique can be utilized for decontamination to reduce the food losses or as quarantine treatment of agricultural products to protect horticultural export against chemical fumigants by improving the environment and hygienic quality. However, among the important benefits of irradiation, destruction or reduction of pathogenic bacteria in foodstuffs is of most importance. In the long term, food irradiation may be seen as a part of the food processing technology to provide food which stays fresher for a longer period.

In food irradiation, which is merely a physical process, the ionizing energy only interacts with the outer orbital electron of the atoms and it does not interact with the nucleus. Consequently, there is no radioactivity induced in the irradiated foodstuffs. The changes only occur chemically in the food without any nuclear changes (Becker, 1983). Irradiation does not cook the foodstuffs since no heat is produced and the food retains its initial characteristics (Satin, 1993). It must also be pointed out that such reactions do not
lead to the formation of aromatic rings, condensation of aromatic rings, and formation of heterocyclic rings (Simic, 1983; and Diehl, 1990).

Since nutritional adequacy and wholesomeness of irradiated foods are important considerations in ascertaining feasibility of irradiation technology, changes in chemical components or nutritional significance have received much attention. However, it has been generally observed that complex foodstuffs are less susceptible to irradiation induced changes in chemical structure of the components as expected from studies using pure compounds (Adam, 1983; and Delincee, 1989). The reaction mechanisms occurring in irradiated foods must be fully understood if consequences of irradiation are to be predicted.

Radiation sources and dosimetry technique are playing important roles in food irradiation. There are 2 types of radiation sources which can be used, i.e., radionuclides and particle accelerators. Some radionuclides emit a form of electromagnetic wave energy called photon. Co-60 or Cs- 137 provides photon, rays, with the energy sufficient for good penetration but unable to induce radioactivity in the irradiated
foodstuffs. Particle accelerators provide a beam of high energy as charge particles. Electron beams are used for radiation processing since the energy is limited to 10 million electron volts (MeV) avoiding traces of radioactivity which may be induced at higher energy.

Several parameters need to be considered in determining the Co-60 radiation dose received by a package, i.e., amount of Co-60 in the source plaque, distance between source and irradiated products, the exposure time, and radiation absorption within the packages which increases as the package density and the package 11 width increase. It is important to minimize dose variation within each package when food is irradiated. Such variation are inevitable because the distance to the source varies from point to point within the package and because of radiation absorption within the package. The ratio of maximum to minimum dose ($D_{\text{max}}/D_{\text{min}}$), designated as uniformity ($U$), must be kept within reasonable limits, usually less than 2. $D_{\text{min}}$ must be sufficient to achieve the objective of the process. The $D_{\text{max}}$ must be within the tolerance of the commodity. $D_{\text{max}}/D_{\text{min}}$ can be minimized
partly by moving the packages around the source during the irradiation so that position of high and low dose is interchanged. The energy absorbed per unit of time is called the dose rate.

The new unit of radiation, Gray, as the amount of irradiation energy that a food absorbs is measured in units. It is noted that 1 Gray = 1 joule per-kg of absorbed energy = 100 rads (the old radiation unit) while at medium irradiation dose, 10 kGy, the energy is equal to to the amount of energy required to raise the temperature of water by 2.4°C.

It is considered that gamma irradiation is applied for different purposes for preserving foodstuffs, e.g., inhibition of vegetables sprouting is (up to 0.2 kGy), insect disinfestation (0.2-1.0 kGy), parasite elimination (up to 6 kGy), microbial load reduction (0.05-5 kGy), killing of non sporin pathogens (3-10 kGy), and absolute sterilization (50 kGy) (Diehl, 1990; and Satin, 1993).
E. Irradiation of Carbohydrate and its Derivatives

The most important effects of ionizing radiation on macromolecules are degradation and cross linking. The degradation process is merely a hydrolysis of glycosidic bounds and other weak linkages yielding simpler compounds (Charlesby, 1960; Charlesby, 1987; and Sonntag, 1992).

Fundamental study on radiation chemistry of different foodstuffs is identification and measurement of various species formed during and after irradiation. Chemistry of the species in the reactions with different compounds covered reaction kinetics, mechanisms, intermediate and stable end products (O'Donnell and Sangster, 1970; and Taub, 1983). An observation on the rate constant of transient species can be done using pulse radiolysis, ESR (Yang et al., 1987) and in combination with competition kinetics (Raffi and Agnel, 1983). In principle ESR can detect the spin unpair electrons even in a very low concentration and the amplitude of ESR signal is approximately proportional to the total number of the electrons in the sample (Wertz and Bolton, 1972). Thus, ESR is
obviously useful for detecting and characterizing free radicals formed in the irradiated samples.

The radiation chemistry of carbohydrate can be determined by three major factors, i.e., the radiation chemistry of the simple sugar sub-units; the effect of substituents on the sub-units; and the fate of glycosidic linkage (Diehl, 1990). The splitting of glycosidic linkages in carbohydrate molecules is not only caused by gamma irradiation but also by enzymes action or by chemical means with some differences in the results. These differences depend on the degree of selectivity among bond cleavages. When carbohydrate as a complex matrix of macromolecules interact with ionizing radiation ionized molecules are produced which immediately react further to form some new products called free radicals. These free radicals can be produced in several micromolar concentration in time periods of a few nanoseconds (Schwarz, 1981). These free radicals continue to react each other and to other compounds leading to new stable chemical radiation products. The life time of those free radicals strongly depend upon several factors such as temperature and the presence of oxygen (Nawar, 1983).
Another theoretical explanation of the depolymerization of high molecular weight carbohydrate has been proposed by Scherz (1971). He postulated that in the solid state the free radicals react with the oxygen of the glycosidic linkage forming a $-\text{O--}$ radical. In the following step the O-C linkage next to the hexose split off forming a positive charge at the C$_4$ atom. This positive ion reacts with the OH$^-$ from irradiated water which is always present either as natural moisture or as solvent. Basically similar to that explanation has also been suggested by Taub (1983), Simic (1983), and Diehl (1990).

When water is present in the sample during irradiation, carbohydrate react primarily with hydroxyl radicals and abstracts predominantly the hydrogen of the C-H bond. The resulting radicals will react further through different mechanisms which is referred as the secondary effect reaction leading to the final formation of products such as acid, keton and aldehyde depending upon the position of C=O bond formed after the reaction (Sonntag, 1980; Simic, 1983; Diehl, 1990; and Anonymous, 1994).
When oxygen is present during irradiation of carbohydrate, the formation of deoxy compounds is suppressed and the yield of sugar acid and keto-sugars will increase with increasing dicarbonyl sugar. The life time of free radicals depend on several important factors such as the presence of oxygen, pH, moisture content of substrate, and temperature during irradiation (Simic, 1983).

The effect of gamma irradiation on degradation pathways of foodstuffs containing water, and its simpler carbohydrate compounds are illustrated in 3 different figures respectively. Figure 1 describes the mechanism of water radiolysis (Taub, 1983; and Diehl, 1990). The stable end products of water radiolysis are hydrogen and hydrogen peroxide even they are largely consumed but the yield is quite low. During irradiation of foodstuffs containing water, the presence of two reactive species, i.e., OH⁻ is an oxidizing agent, and e⁻aq as reducing agent should be considered.
\[ \text{H}_2\text{O}^+ \rightarrow \text{H}_2\text{O}^+ + \text{e}^- \text{aq} \quad \text{ionization} \quad (1) \]

\[ \text{H}_2\text{O}^* \quad \text{excitation} \quad (2) \]

\[ \text{deprotonation/ proton transfer reaction} \quad \text{H}_2\text{O}^+ + \text{H}_2\text{O} \rightarrow \text{H}_3\text{O}^+ + \text{OH}^- \quad (3) \]

\[ \text{solvated proton} \]

\[ \text{H}_2\text{O}^* \rightarrow \text{H}_2\text{O} \quad \text{deexcitation} \quad (4) \]

\[ \text{H}_3\text{O}^+ + \text{OH}^- \rightarrow \text{H}_2\text{O}^* + \text{H}_2 \quad \text{dissociation} \quad (5) \]

recombination (6a) and combination reactions (6b):

\[ \text{H}_3\text{O}^+ + \text{OH}^- \rightarrow \text{H}_2\text{O} \quad (6a) \]

\[ \text{e}^- \text{aq} + \text{OH}^- \rightarrow \text{OH}^- \quad (6b) \]

\[ \text{e}^- \text{aq} + \text{H}_3\text{O}^+ \rightarrow \text{H}_2\text{O}^+ + \text{H}_2 \]

\[ \text{e}^- \text{aq} + \text{e}^- \text{aq} + 2 \text{H}_2\text{O} \rightarrow \text{H}_2 \quad +2\text{OH}^- \]

\[ \text{OH}^- + \text{OH}^- \rightarrow \text{H}_2\text{O}_2 \quad \text{dimerization} \quad (7) \]

\[ \text{H}_3\text{O}^+ + \text{OH}^- \rightarrow \text{H}_2\text{O} \quad (6a) \]

\[ \text{H}_2 \text{O}^+ + \text{H}_2 \text{O} \rightarrow \text{H}_2 \]

**Figure 1. Reaction mechanism of water radiolysis**

(Diehl, 1990)
When glucose as a simple model for carbohydrate is irradiated in solute state, water radiolysis generates OH radicals which abstract predominantly hydrogen of C-H bonds at position C-1 resulting acid, aldehyde or ketone (Figure 2) (Diehl, 1990).

Through loss of CO the 6-carbon sugar glucose can also be converted to the 5-carbon sugar arabinose:

Ring opening can lead to 5-deoxy gluconoic acid:

Figure 2. Degradation mechanism of irradiation glucose (Diehl, 1990)
Gamma irradiation induced degradation both as direct and indirect effects on aliphatic polyhydric ether compound (RCHOHCHOHOR') as a structure of aliphatic carbohydrate representative is illustrated in Figure 3 (Taub, 1983).

When an aliphatic-polyhydric ether compound representing a simpler carbohydrate is irradiated by gamma rays a continuous chain reaction along the molecules will occur through either direct or indirect effect depending on the substrates.

Many products in the radiolysis of organic substrates and radiolytic of aromatic systems after radiation exposure at a very low dose can be determined in the range of micromolar levels using HPLC methods involving spectrophotometric detection with diode array detectors (Schuler, 1992).
Figure 3. Direct and indirect effect reactions on irradiation of aliphatic polyhydric compound (Taub, 1983)
ESR experiments have been carried out by other investigators in order to elucidate the mechanism of production of free radicals from irradiated carbohydrate containing starches, fruits and vegetables (Desrosier and Laughlin, 1986) and spices. The ESR study of irradiated pepper and cinnamon revealed that decreasing in free radical concentration indicated by the reduced value signal was due to the type of samples, irradiation dose and storage time (Sudiro et al., 1994).

Effect of irradiation on the development of radiolytic products in other carbohydrate members such as starch polysaccharides derived from different foodstuffs such as spices, grains, and cereals have also been intensively studied in many countries.

The test which is proposed for the proof of irradiated starch is based upon the determination of malonaldehyde and other radiolytic compounds produced by irradiation. The changes in the level of induced radiolytic products were analyzed as a function of irradiation condition (dose, dose rate, temperature and atmosphere), starch properties (water content, and pH) and post irradiation treatment or

An experimental work using different types of irradiated starches such as maize (MN), amylomaize (AM), waxy maize (WM), bread wheat (B), manioc (M), rice (R), potato (P) and haricot bean (H) has been carried out by Raffi$^a$ et al. (1981); Raffi$^b$ et al. (1981); Raffi$^c$ et al. (1981), and Raffi$^d$ et al. (1981). Irradiation was conducted under 3 different conditions, viz. air, nitrogen, and oxygen. Samples were irradiated with doses ranging from 5 to 30 kGy at 25°C to study radiation induced formation of radiolytic products, and irradiation at 10-60 kGy for depolymerization study and kinetic law of the radicals. The parameters observed were total carbonyl derivatives (2,4-dinitrophenylhydrazine method), malonaldehyde (thiobarbituric acid test), formaldehyde (phenylhydrazine hydrochloride) and hydrogen peroxide (ammonium thiocyanide with iron [II] sulfate); gas chromatography for formic acid (under methyl formate form) and acetaldehyde. Total acidity was measured by pH-metry, gravimetry for water soluble dextrins and reducing power was measured under back-
titration of iron [II] cyanide method. The spectra were recorded at room temperature on a spectrophotometer coupled with computer programme. The result shows that at water content equilibrium the quantities of carbonyl derivatives radioinduced in the different starches always have the same order of magnitude: \( r \) varies from 2.1 (total carbonyl derivatives) to 4.6 (malonaldehyde) while intrinsic viscosity has strong correlation with the average degree of polymerization.

Michel et al. (1977) reported that the percentage of identified and unidentified carbonyl fraction resulting from irradiated maize starch were 40 and 60%, respectively. Half of the unknown fraction was linked to the radio dextrins.

Formic acid presence in the irradiated maize starch was observed (100 \( \mu g/g/Mrad \), in oxygen) (Dauphin et al., 1974).

Research on the formation of glycolaldehyde in irradiated maize starch has been done by Hamidi and Dauphin (1976). They reported that in irradiated maize starch glycolaldehyde was found (5.6 \( \mu g/g/Mrad \), in oxygen).
Different types of sugars with low molecular weight derived from irradiated maize starch have been identified and determined using ion exchange chromatography. The samples were treated in different conditions, i.e., irradiation atmosphere, starch properties, and storage. Different type of sugars were found such as one saccharide (maltose), and hexoses (glucose, mannose, galactose, fructose). Pentoses such as arabinose, xylose and ribose were also found and also slight uncertainty amount of tetrose/erythrose (Berger et al., 1973).

Studies on the formation of glyceraldehyde (G), dihydroxyacetone (DHA) and 2-hydroxymalonaldehyde (H2M) in maize starch (amylopectin rich) using NMR and gas chromatography method have been carried out by Raffi et al. (1981). They obtained that the concentration of the radioinduced products increased in a linear manner with the dose up to 50 kGy. (G=0.49 μg/g/kGy; DHA=0.12 μg/g/kGy; and H2M=0.62 μg/g/kGy). The total quantity slightly decreased when the samples were irradiated under nitrogen atmosphere. Storage condition, in the presence of oxygen at room temperature, could reduce the amount of
those products rapidly particularly in the first week. They concluded that generally the radio induced quantities of G, DHA, and \( \text{H}_2\text{M} \) are too low to involve health hazards. For industrial purposes, the synergistic effect was obtained when proton was combined with gamma radiation.

Further experimental work on the same subject indicated that Raffia et al. (1980), and Raffib et al. (1980) have found a mathematical model making possible to calculate the value of intrinsic viscosity and the rate of water-soluble products of "acidified-irradiated" starch as a function of: irradiation dose, total time of acid hydrolysis, proton and water content of the starch. Furthermore, depolymerization of starch in presence of acid under irradiation results in a general drop of the braebender viscosity as expression of rheogram prediction (Michelc et al., 1980). Besides, other factors such as concentration of starch paste and temperature will influence the intrinsic viscosity of the irradiated starch. Finally they concluded that this study is possible to be applied for dextrinification. Furthermore irradiation sample under acid hydrolysis is not important, and combination treatment between proton, and
gamma radiation can lead to a synergistic effect on the formation of water soluble products. The possible glucosidic bonds cleavages are only due to water content of starch, and not caused by the position of nature branching which exist on the position-1,4 or -1,6 (Michel et al., 1980).

Other study on radiodepolymerization of starches has been carried out by Michel et al. (1980). Three types of starches, i.e., amylomaize, waxy maize and normal maize, both unirradiated and irradiated with the dose below 50 kGy were suspended in water (max. 3% conc.) brought to pH 5 and heated to 95°C for 20 min. The suspended sample were then diluted into 1% conc. using soda solution. The physicochemical properties of macromolecular part and water soluble fraction of starches measured were intrinsic viscosity, reducing power, total glucose or glucose equivalent, rate of water soluble products and average degree of polymerization. The results revealed that the inverse intrinsic viscosity of different starches increased linearly with increasing irradiation dose. They have found that irradiation at 6.2 kGy caused one cleavage per
molecule on average, i.e., one cleavage of bond about ten million bonds.

A GC/MS method has been applied for identification of some volatile compounds such as acetaldehyde, methanol, acetone, small amounts of methyl formate and various quantities of ethanol which found in irradiated corn starch (Berger et al., 1974).

Effect of gamma irradiation on the modified corn starch and fine flour with acid has also been studied in Egypt by many investigators (Ghali et al., 1979). They concluded that the C-2 and C-3 bonds in the intermediate glucose units are probably broken by irradiation. This result is proved by the parallel experimental works on periodate oxidation, hydrolysis with amylase and ferricyanide number determination of the irradiated strach.

An experimental work on the effect of high doses of gamma irradiation on corn grains was carried out by Roushdi et al. (1981), and Roushdi et al. (1983). They proposed that high irradiation doses cause a high release of energy resulting in the cleavage of the glycosidic and peptide linkages, respectively. The released energy could
also cause the interaction between liberated carbonyl and amino compounds developing a colour as in Maillard reaction. Irradiation with doses of less than 50 kGy could extend the shelf life of grains without affecting the starch quality. In other parallel study they also reported that irradiation doses up to 20 kGy could reduce the percentage of amylopectin but increase the percentage of amylose starch and its fractions of corn grains. The chain length both in amylose and amylopectin were reduced by irradiation with doses higher than 20 kGy. Other analytical method such as infra red analysis was used in the same experiment. They proposed that the instrument is a powerful tool to provide a rapid indication regarding the composition of the carbohydrate. The absorption or the lack of absorption at specific frequency regions could be correlated with specific functional groups.

Different low-doses of irradiation were subjected to corn starch resulting in the formation of malonaldehyde. Improvement of its detection method was also intensively studied by Winchester (1973); Winchester (1974); Stewart and Winchester (1975), and Winchester (1976) from South
Africa. They found that moisture content and irradiation temperature would influence the formation of malonaldehyde. It is an indication that results obtained from infra red spectra, NMR spectra and the elemental analysis shows similarity of structure between synthesized compound and the compound derived from starch. Hydrogen peroxide did not react with malonaldehyde when they were mixed into unirradiated starch. When amino acid was added into unirradiated starch containing malonaldehyde, the malonaldehyde dissapeared at a measurable rate. It seemed that the disappearance of malonaldehyde as a radiolytic product in irradiated starch was due to the reaction between malonaldehyde and amino acid or malonaldehyde and other starch radiolytic products (Winchester, 1976).

Other study on the radiodepolymerization of corn starch for the surface-sizing paper industrial purposes was investigated by Hofreiter et al. (1974).

El-Saadany et al. (1979) mentioned that gamma irradiation caused degradation of starch molecules followed by a reduction of viscosity and swelling capacity of rice
starch and an increase in their soluble constituent. These parameter changes have a correlation with the reduced cooking time and increasing softening properties. They have done the similar experimental design on irradiated corn starch. The result revealed that irradiation with doses from 1-10 kGy could reduce the gelatinization time, temperature, and viscosity but increase the starch acidity and reducing power (El-Saadany et al., 1976).

Study on the low molecular degradation products due to gamma radiation with the dose of 50 kGy on potato starch was done (Scherz, 1971). An extraction followed by two dimensional thin layer chromatography method were applied and resulting in 16 substances. Then they classified the polysaccharides into different components using color reaction, i.e., monosaccharides, deoxycompounds, sugar anhydrides and furan. The gas chromatographic separation of the trimethylsilyl ethers of the substances obtained after reduction of the extract with potassium borohydride gave approximately 25 compounds to be analyzed by mass spectra. Evaluation upon position of carbonyl groups and mass shifting using mass spectra indicated that aldoses,
deoxysugars, deoxycarbonylsugars, and cyclic compounds were found in irradiated potato starch.

Studies on the effect of gamma irradiation on egyptian sweet potato starch revealed that in the irradiated samples with doses ranging from 5 up to 10 kGy, a marked increase in starch acidity and reducing sugars as well as decreasing in molecular weight were indicated by the decreasing viscosity and water absorption and increasing in solubility (Raouf et al., 1974; and Rhousdi et al., 1982).

Other analytical method such as electron spin resonance was applied by Raffi and Agnel (1983) to investigate the influence of physical properties of 8 irradiated starches during storage. Irradiation was done under nitrogen at room temperature with the dose varied from 5 to 20 kGy. They emphasized that ESR spectra showed poor resolution in starch which was irradiated in powder form. They suggested that it is necessary to carry out an experiment on radiolysis of simple sugars both in powder state and monocrystal using spin trapping techniques. Furthermore it is found that radical concentration form in this experiment was too small to induce toxicity in irradiated starch. These radicals are
destroyed immediately when the irradiated (powder) starches are dissolved.

It can be explained that based upon the relative transparency of starch to gamma irradiation, and the cage effect predominant a combination reaction among 2 free radicals after the cleavage of the structural bonds take place. The most important radio-induced modification is the depolymerization of the starch and not the formation of toxicological products of low molecular mass (Raffi et al., 1981).

Different studies on gamma radiolysis of different rich-starch foodstuffs such as acid, carbonyl derivatives, and radiodepolymerization irradiated at room temperature under oxygen has been done by Raffi et al. (1981). They observed that total quantity of carbonyl derivatives, malonaldehyde, and formaldehyde linearly increases with increasing irradiation dose from 0 up to 20 kGy. The ratio maximal and minimal in irradiated starches, with the dose up to 30 kGy, induced acidities found the value of 2.5 for formic acid and 3.2 for total acidity (Raffi et al., 1981) while has a
little influence upon the quantities of gamma radio-induced carbonyl derivatives.

Different starches were subjected to irradiation with doses from 0 up to 30 kGy was to study radio-induced carbonyl derivatives using color methods (2,4-dinitrophenylhydrazine), malonaldehyde (thiobarbituric acid), formaldehydes (phenylhydrazine hydrochloride), hydrogen peroxide (ammonium cyanide), with iron II sulphate, formic acid (methyl format form) and acetaldehyde by gas chromatography. Total carbonyl groups and malonaldehyde formation showed a linear increase with increasing irradiation dose. Moisture content of samples gave strong influence in production of total carbonyl groups and the presence of oxygen in dry state played an important role in malonaldehyde formation than in the moist state. The state of water (free, associated or linked) is the main physical factor influencing the formation.

Formation of formaldehyde from different starches was strongly influenced by the presence of water and oxygen. Generally, the formation had a linear increase with increasing irradiation dose. The same result was obtained
in the quantity of acetaldehyde formation from irradiated maize, amylomaize, waxy maize, rice and potato but the peak showed a decline in certain irradiation dose.

Study on acid formation resulted that formation of formic acid due to irradiation with the dose of 20 kGy reached maximum peak and the presence of oxygen showed a strong influence during the formation except in waxy maize. Other study on radiation induced free acidity formation of starches resulted that irradiation with the dose ranging from 5-20 kGy showed a linear increase. A parabolic shape was also found in the irradiated starches with the dose of 10 kGy. Free acidity formation in irradiated maize starch, amylomaize, manioc and bread wheat with respective dose of 15 kGy was due to formic acid. In waxy maize, the formation of free acidity due to the same acid was only 40-50%. The determination of formation of total acidity using titration method from the irradiated starches showed that the formation was less influenced by the presence of water but strongly the affected by presence of oxygen.
The mechanism of starch radiolysis using glucose and glucose oligomers with DP 4.5 and 6, respectively, as model systems has been investigated by Raffi et al. (1985). They obtained that malonaldehyde is known to be produced by irradiation with the dose up to 40 kGy, for both glucose and starch. Their quantitative determination can be used as a test of irradiated starches. The main radiolytic product of irradiated starch at doses up to 15 kGy is formic acid. The physical state of different type of sugar will also give strong influence on the resulting products as well as yield of radiolytic products.

Another study which deals with the shape and kinetic changes of the ESR spectra of different irradiated starches at several storage time has been done by Raffi et al. (1983). They concluded that depending on the origin and water content of the samples two major radical groups were observed. The change in kinetic spectra depends upon the water content of samples. Two main zones were pointed out which were relative to the amorphous and crystalline part of starches. The starches in powder form resolved very poor. It is necessary to carry out the experiments on radiolysis
simple sugars, not only in powder but also in monocystal forms using spin trapping technique, because the toxic radicals can not be detected from dilute irradiated starches in water prior to analysis.

Other study on radical formation irradiation of dried products has been conducted (Yang et al., 1987). Dried spices and spray dried fruit powder were irradiated with the dose of 30 kGy at 30°C radiation-atmospheric temperature. The result revealed that the paramagnetic signal from ESR was not affected by the presence of oxygen but the radicals decayed rapidly in water.

Other studies concluded that in dry and solid materials some of the highly excited state of valence electrons may be fairly stable. Dry sugar crystals or other polysaccharides can have long lived excited states. In some of these states, they may have some unpaired trapped electrons or electron holes which can be detected by ESR. The trapped energy can probably be released by electromagnetic waves, heating or dissolving the sample in water or other liquid. The released energy which is caused by chemical reaction can
be detected by thermoluminescence (TL) for heating and
lyoluminescence (LL) for the liquid.

In searching a reliable detection method to identify
irradiate food particularly irradiated spices chemilumines-
cence (CL) and TL have been widely used by many
investigators. Sjoberg et al. (1990) and Sjoberg et al. (1991)
reported that the best method to identify irradiated spices
for controlling purposes were microbiological methods
(epifluorescence filter technique and a total aerobic plate
count) combined with chemiluminescence measurement.

Detection of irradiated corn starch using low-dose of
radiation was briefly reported by Stewart and Winchester
(1973). The rate of malonaldehyde formation in irradiated
corn starch at doses up to 1 kGy and stored at 23°C for 3
weeks depended upon the moisture content of the sample
and temperature during irradiation. Different storage
condition (temperature and time) did not influence the
formation. A follow up study of the same authors on positive
identification of malonaldehyde in irradiated starch by
isolation and characterization of the thiobarbiturate
derivatives resulted that the synthesized compound and
compound derived from irradiated starch with doses of 4 and 8 kGy showed the same structure as in physical determination by infra red, NMR spectra and elemental analysis. The malonaldehyde from irradiated starch was measured by thiobarbituric acid test but the result was rather doubtful.

An ion exchange chromatography was applied to identify and to determine the formation of sugars with low molecular weight from irradiated maize starch with the dose of 20 kGy.

The resulting sugars were one disaccharides (maltose), hexoses (glucose, mannose, galactose and fructose), pentoses (arabinose, xylose and ribose) and one tetrose (erythrose). A slight uncertain result remained in the case of mannose and erythrose production.

A systematic study on the chemiclearance of irradiated different starches, i.e., maize, amylo maize, waxy maize, bread wheat, manioc, rice, potato and haricot bean has been carried out by Raffiò et al. (1981).

The effect of pH of medium, structure and organization of starch macromolecules in which oxygen can infiltrate
the molecules with different velocity might play an important influence upon peroxide formation in irradiated starches. It was concluded that maize starch was a good model for carbonyl acid radio-formation by the ratio: \( \frac{R}{R_{mn}} = 0.68 \) (\( R = \) average value from all different starches and \( R_{mn} = \) value of maize).

Irradiated maize with the dose up to 50 kGy showed a linear increase in the formation of glyceraldehyde, dihydroxyacetone and 2-hydroxymalonaldehyde. Temperature and the presence of oxygen during irradiation gave strong influence on different formation. The formation of these radiolytic products decreased with increasing storage time. Duration in storage period would influence the analytical results in the study on irradiated straches. It is suggested that the experiment has to be conducted within one week period since the equilibrium of water content is very sensitive to the atmospheric changes.
III. MATERIALS AND METHODS

Sample preparations, and method of analysis were selected according to three different experimental stages, i.e., first experimental study using irradiation of isolated cell wall polysaccharides of mango flesh to determine their degradation products. Mango as climacteric-tropical fruit which is rich in nonstarch polysaccharides constituents was considered to be used as starting raw material. This fruit has been chosen as a model due to their compositions, and to provide more information on certain chemical changes of the isolated wall after irradiation with the presently permitted maximum dose for treating food (Anonymous, 1981). Second experimental work using less complex materials related to the wall components indicated as superimpose model systems which covered anionic and neutral cell wall polysaccharides with variation in the degree of branching (arabinans), and of variation in sugar building units such as degree of substitution with ester groups (pectins and alginates). These type of sugars were considered to be used as model systems to mimic cell wall components originated from polysaccharides which are present
in mango flesh. Alginites in different types of sugar building blocks were also used in this experiment to distinguish their stabilities against irradiation treatment; Third stage of experiment was undertaken to study in more detail the effect of gamma irradiation on simpler compounds presented as intermediate oligomers. Oligomers with different degree of polymerization were hydrolized and depolymerized into some chemical entities, then subjected to gamma irradiation and producing new fragments.

A. Effect of Gamma Irradiation on Cell Wall Polysaccharides of Mango (*Mangifera indica* L.) var. Tommy Atkins

It is very difficult to measure the effect of irradiation on mango fruit as complex tissues. Various isolation methods have been selected in order to have some information on the degradation products of irradiated cell wall polysaccharides. The effect of irradiation on the isolated cell wall was conducted after sequential extractions to obtain soluble pectin, hemicellulose and cellulose.
1. **Preparation of cell-wall material**

Mango (*Mangifera indica* L.) var. Tommy Atkins at commercial maturity stage (1.3 kg pulp) was used for the extraction of cell-wall material. The Alcohol Insoluble Solids (AIS) was prepared by precipitation of the wall in alcohol (ethanol) according to Heutink (1986) followed by washing the mango tissue with 70% ethanol until sugars free was achieved in the AIS samples.

2. **Experimental design and sampling methods**

The statistic method used was complete randomized design in triplicates according to Steel and Torrie (1981), and a diagonal sampling method as described by Lees (1971). To study radiation induced-degradation on solute polysaccharides in vitro, AIS was partly suspended in water (1:1) stirred, and kept over night at room temperature prior to irradiation.

3. **Irradiation treatment**

AIS as Non-suspended (powder) and as suspension, were irradiated under presence of oxygen at 20°C by the Co-60
conducted at the Institute for Atomic Sciences in Agriculture (ITAL) Wageningen, The Netherlands. A dose of 10 kGy was applied at a dose rate of 6.3 kGy/h.

Powder and AIS in water before and after irradiation were subjected to extraction by Na$_2$CyDTA solutions. Partially depectinated cell wall, viz. Na$_2$CyDTA Insoluble Residue (CyDTAIR) and Na$_2$CyDTA Soluble Pectin (Na$_2$CyDTASP) preparations were obtained as described by Renard et al. (1990). These solutions were adjusted to pH 4.8 - 5 to prevent β-eliminative degradation of pectic material during extraction. CyDTAIR were further fractionated by extracting sequentially with 50 mM NaOH solutions, 4 M NaOH solutions containing 0.26 M NaBH$_4$ to yield Alkali Soluble Pectin (ALKSP), a fraction rich in hemicellulose, and a cellulose rich residue (Voragen et al., 1983; and Vries et al., 1981). Figure 4 shows a detailed extraction scheme.
FRESH MANGO

(Mangifera indica L.) var. Tommy Atkins (Fruit pulp)

↓ Alcohol Insoluble Solids (AIS)

↓ Non suspended (powder) AIS

↓ Suspended in H₂O (1:1) AIS

↓ control (irradiated (10 kGy))

↓ control (irradiated (10 kGy))

Extracted by 0.02M Na₂CyDTA pH 4.8-5.0

↓ 6 h at room temp (± 25°C)

↓ centrifuged 27300 g/20 min at 5°C

Na₂CyDTA Insoluble Residue washed

with water, freeze dried & weighed

→ as fraction: CyDTAIR

↓ 50mM NaOH 1 h at 0°C

Centrifuged 27300 g/20 min at 5°C

Pellet → reacted with:

0.26 M NaBH₄ in:

4.0 M NaOH 8 h at ± 25°C

↓ centrifuged

Pellet → reacted with:

72% w/w H₂SO₄

↓ filter

↓ lignin

↓ cellulose

Supernatant dialyzed, freeze dried & weighed

→ as fraction: Na₂CyDTASP

Supernatant dialyzed, freeze dried & weighed

→ as fraction: ALKSP

Supernatant was then acidified to pH 5.5 dialyzed, freeze dried & weighed

→ as fraction: hemicellulose

Figure 4. Extraction scheme of cell wall polysaccharides in mango (Mangifera indica L.) var. Tommy Atkins.
4. Analytical methods

a. Cellulose content

AIS and of CyDTAIR were extracted by strong acid
followed by an anthron reaction to measure cellulose
content according to Up de Graaff (1969) method.

b. Uronic acid content

Uronic acid content in samples was determined by the
colorimetric m-Hydroxyl diphenyl assay by Ahmed and
Labavitch (1977) using anhydrogalacturonic acid solutions
as standard.

c. Neutral sugar analysis

Prior to the chromatography measurement the fraction
was first treated with aqueous 72% w/w H₂SO₄, for 1 h at
30°C, followed by hydrolysis with 1 M H₂SO₄ (3 h at
100°C) and conversion of the individual sugar residues
into alditol acetates. Inositol was used as internal
standard (Englyst and Cummings, 1984).

d. Gas Liquid Chromatography (GLC)

The alditol acetates were analyzed on a glass column (3
mm x 2mm i.d.) packed with Chromosorb W-AW 80-100
mesh coated with 3% OV 275, using a Carlo Erba Fractovap 2300 GC operated at 200°C and equipped with a FID detector set at 270°C. Nitrogen was used as carrier gas (Albersheim et al., 1967).

e. High-performance ion exchange-chromatography (HP-IEC)

The elution behaviour of the charge polysaccharides on an anion-exchange column was studied as described by Schols et al. (1989). A Biorad MA7P column (50x7.8 mm) was eluted with a linear gradient of 15-270 mM using Na-phosphate buffer at pH 6.0 at a flow rate of 1.5 ml/min. Detection was performed using a diode array detector by monitoring the absorbance at 215 nm. The increase in baseline signal was corrected by subtracting the chromatogram obtained for a blank run from those of sample runs.

f. High-Performance Size-Exclusion Chromatography (HPSEC)

The experiment was performed on a SP 8800 HPLC system (Spectra Physic, San Jose, USA), equipped with 3 Biogel TSK
columns (300 x 7.5 mm) in series (60XL, 40 XL, and 30 XL; Biorad Labs) in combination with a TSK XL guard column (40 x 6 mm) and elution at 30°C with 0.4M acetic acid/Na-acetate (pH 3.0) at 0.8 ml/min. The eluent was monitored by a Shodex SE-61 Refractive Index Detector. Calibration of molecular weight was done against pectin standards with molecular weights ranging from 10,000 - 100,000 Dalton as determined by viscosimetry. Samples (3-5 mg/ml) were solubilized in 0.4 M Na-acetate buffer at pH 3 and centrifuged prior to injection.

B. Effect of Gamma Irradiation on Polysaccharides Model

**Systems**

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 extremely 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
extremely difficult (Simic, 1983). Therefore a study of specific features of the degradation can only effectively be carried out utilizing less complex systems (Diehl et al., 1978; Raffi et al., 1985; Drijver et al., 1986; Sjoberg, 1987, and Beyers, 1983). Polysaccharides such as pectins, and alginates are considered useful model systems polymers to study the degradation of polysaccharides with structural variations by irradiation in vitro.

I. Polysaccharides model systems

A commercial high methoxyl apple pectin (HMP) with a degree of methylation (DM) of 76%, and pectic acid (DM = 2%) were 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). Two chemically modified alginates, viz. propylene-glycol type 109 (highly viscous), and type 110 (low in viscosity) were also obtained from Kelco.
Highly branched arabinan (HBA) from sugar beet (degree of branching >10%) (Voragen et al., 1987) as well as a linear arabinan extracted from apple juice were obtained as described by Rombouts et al., (1988).

2. Sample preparation

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 Büchii 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$_2$SO$_4$ test as described by Dubois (1958). The alcohol insoluble residue of HMP was finally rinsed with 96%
ethanol. The insoluble residue was dried by solvent exchange (acetone followed by ether) and drying at room temperature for 3 days. The dried material was pulverized to a fine powder (0.7 mm) using a Cullatti DFH hammer mill prior to its use.

The alginates M/G 2.5 and M/G <1 were transferred to the acid form according to the method in Food Chem. Codex (1981). Samples of 5 g were transferred into beakers and stirred for 10 min with a mixture of 5 ml concentrated hydrochloric acid and 100 ml of 60% isopropyl alcohol. The mixtures were then centrifuged at 39,900 g for 20 min. The alcohol insoluble residues were washed 6 times with 15 ml portions of 60% isopropyl alcohol until the filtrates were chloride free. The residues were dried at room temperature by solvent exchange, i.e., acetone and ether. The 2 alginic acid samples were neutralized by dissolving in 0.05 N NaOH (0.4 ml/2 mg sample) prior to analysis.

Highly branched arabinan (HBA) was used in powder form after conditioning and as solution after dissolving in distilled water at a concentration of 2 mg/ml.
3. Conditioning of samples

Different humidities were set according to Labuza (1984) and Iglesias and Cherise (1982) using saturated solutions of two different inorganic salts, e.g., K$_2$CO$_3$ and NaBr providing relative humidities (RH) of 40.3% and 58.1% respectively. Each salt solution was placed on the bottom of a desiccator and an equilibrium was allowed to form under vacuum (10 mg Hg) over night at 23°C. The polysaccharides (1-3 g) were kept at 23°C in the prepared dessicators having a known relative humidity until the weight of the samples became constant. The moisture content of equilibrated samples was determined using a Karl Fisher automatic titrator.

4. 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%. Some samples were also irradiated in solubilized form (2mg/ml distilled H₂O) with doses of 1, 3, 5 and 10 kGy. Samples of dissolved HBA partly received doses of 1, 3, 5, and 10 kGy under oxygen and other dissolved samples were irradiated with doses of 5 and 10 kGy after purging with nitrogen. Samples of linear arabinan, i.e., type 50 was dissolved in distilled water (2mg/ml) and irradiated with doses of 5 and 15 kGy in the presence of oxygen. Non-irradiated samples of the various polysaccharides kept at comparable conditions served as control.

5. Analytical methods

a. Viscosity

Viscosity was measured in an Ubbelohde capillary viscosimeter as described by Deventer-Schriemer and Pilnik (1987).
b. 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 (1972).

c. High-Performance Size-Exclusion Chromatography

(HPSEC)

Estimation of molecular weight was performed on a SP
8800 HPLC (Spectra Physics) equipped with three Bio-gel
TSK columns (300 x 7.5 mm) in series (60 XL, 40 XL, and
30 XL; 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
(Schols et al., 1990). The eluate was monitored by a
Shodex SE-61 Refractive Index detector.

The degree of methylation of HMP and the alginic acid
content of the alginate M/G 2.5 and alginate M/G < 1
were determined by a titration method (Food Chem.
Codex, 1981). The degree of methylation expressed as %
total carboxyl groups results from the formula:
Degree of Methylation of HMP(%) = \( \frac{V_2 \times 100}{V_1 + V_2} \)

Alginic acid of alginates (%) = \( \frac{V_1 \times 17.6}{3} \)

\( V_1 \) = first titration volume (ml) \( \times \) conc. NaOH (0.1 N)

\( V_2 \) = second titration volume (ml) \( \times \) conc. NaOH (0.1 N)

176 = molecular weight of anhydrous uronic acid

3 = weight of sample (300 mg)

d. High-Performance Ion-Exchange Chromatography (HPIEC)

The elution behaviour of the intermolecular charge distribution of polysaccharides was conducted chromatographically on an anion-exchange column as described by Schols et al. (1989). A Biorad MA7P column (30 x 7.8 mm) was eluted with a linear gradient of 15-270 mM using Na-phosphate buffer at pH 6.0 at a flow rate of 1.5 ml/min. Detection was performed using a diode array detector by monitoring the absorbance at 215 nm. The increase in baseline signal was corrected by subtracting
the chromatogramme obtained for a blank run from those of sample runs.

e. High Performance Anion Exchange Chromatography (HPAEC)

Analysis on the degradation products was performed on a Dionex Bio-LC system (Sunnyvale, California, USA) including a quarternary gradient pump, eluent degas (He) module, and pulsed electrochemical detector PED) in the pulsed amperometric mode (PAD), completed with a Spectra Physics SP 8800 autosampler and a Spectra Physics Winner data handling system. A CarboPac PA100 column (4x250 mm) with matching guard column (Dionex) was used at a flow rate of 1.0 ml/min. The gradient was obtained by mixing solutions of 100mM NaOH and M NaOAc in 100mM NaOH. After an equilibration step of 15 minutes with 1M Na-acetate in 100mM NaOH, 20 μl of the sample was injected and a linear gradient to 600 mM Na-acetate in 100mM NaOH within 45 minutes was started. Finally the column was washed for 5 minutes with M Na-acetate in 100mM NaOH and equilibrated again for the next injection.
6. *Statistic calculation*

Analysis of variance of the complete randomized design on \( \eta \) viscosity, and DP were calculated statistically according to Steel and Torrie (1981).

C. **Degradation of Fragmented Polysaccharides Induced by Irradiation**

Another approach to study the mechanism of the degradation of glycosidic linkages during irradiation is to use oligosaccharides as model substrates. The great advantage of this strategy is that both the starting oligomers were obtained either by enzymic or by mechanolysis fragmentations as well as their degradation products are within the range of modern analytical techniques such as high-performance anion-exchange chromatography (HPAEC).
1. Fragmentation of polysaccharides

a. Low Mw polygalacturonic acid fragments obtained by enzymic degradation

Polygalacturonic acid (PGA; ICN Biochemicals Inc., Costa Mesa, California, USA) was degraded by endo-polygalacturonase (endo-PG) purified from the culture liquid of the yeast Kluyveromyces fragilis (Versteeg, 1979). Enzyme activity is expressed in unit, one unit being the amount of enzyme which split one µmol of glycosidic bond per minute under defined conditions.

Enzymic degradation was performed by incubation of a solution of PGA (0.5%) in 0.05 M Na-Acetate buffer pH 5.0 containing 0.01% NaN₃ and 20µl of 5% endo-polygalacturonase for 2.5 h at 30°C. After incubation, the enzyme was inactivated by heating the solution at 100°C for 10 min. The digest was analyzed by high-performance size-exclusion chromatography (HPSEC).
b. Low Mw fragments of pectins and alginates obtained by mechanolysis

Low Mw pectin fragments were prepared from an apple pectin with a degree of esterification (DE) of 76% (high-methoxyl pectin, HMP, Obipectin Ltd., Bischofszell, Switzerland) and low Mw alginate fragments were prepared from an alginate with a high mannuronic acid/guluronic acid ratio (M/G 2.5) and from an alginate with a low ratio (M/G <1) obtained from Kelco International Ltd. San Diego, California.

The afore mentioned polysaccharides were first desalted. Mechanolysis (ball milling) was carried out according to the method of Deventer-Schriemer & Pilnik (1987) by transferring the polysaccharides (18 g) into cylindrical jars filled with porcelain cylinders. The jars were vibrated with a Vibratom SM (Siebtechnik GmbH, Muhlheim, FRG) at a frequency of 1420/min and an amplitude of 1.75 mm at room temperature. After every two hours of milling the jars were cooled to 4°C. At certain time intervals within the mechanolysis process, samples were taken and the progress of the degradation was monitored by HPSEC. An average degree of
polymerization (DP) of ca. 20 was obtained after 55 h mechanolysis treatment of HMP and of 84 h for the alginates.

2. Fractionation of the fragmented polysaccharides

These two types of fragments, i.e., fragmented polygalacturonic acid after enzymic treatment, fragmented pectin, and alginates were then fractionated by chromatography methods.

Solutions of the different types of degraded polysaccharides (350 mg) dissolved in water (3 mL) were applied on a column (100 x 2.5 cm) of Sephacryl S100 (Pharmacia, separation range for dextrans 500-30,000 Da) and eluted with a 0.1 M Na-acetate buffer at pH 4.0. Fractions were assayed by automated methods for uronic acid (Ahmed and Labavitch, 1977; and Thilbault, 1979) and total neutral sugars (Tollier and Robbin, 1979).

3. Analytical methods

The analytical methods are characterizing polygalacturonic acid, pectin and alginates fragments
according to their molecular weight distribution to calculate the degree of polymerization, and to indicate types of oligomers.

a. *High Performance Size Exclusion Chromatography (HPSEC)*

Distribution of molecular weight was performed on a SP8800 HPLC (Spectra Physics) equipped with three Bio-Gel TSK columns (300 x 7.5 mm) in series (40XL, 30XL, and 20XL; Bio-Rad Labs) in combination with TSK XL guard column (40 x 6 mm) and elution at 30°C with 0.4 M acetic acid/Na-acetate (pH 3.0) at a flow rate of 0.8 ml/min (Schols *et al.*, 1990). The eluate was monitored by a Shodex SE-61 Refractive Index detector.

b. *High Performance Anion Exchange Chromatography (HPAEC)*

Separation of the degradation products was performed on a Dionex Bio-LC system (Sunnyvale, California, USA) including a quarternary gradient pump, eluent degas (He) module, and pulsed electrochemical detector (PED) in the pulsed amperometric mode (PAD), completed with a
Spectra Physics SP8800 autosampler and a Spectra Physics Winner data handling system. A CarboPac PA100 column (4 x 250 mm) with matching guard column (Dionex) was used at a flow rate of 1.0 ml/min. The gradient was obtained by mixing solutions of 100 mM NaOH and 1 M Na-acetate in 100 mM NaOH. For galacturonic oligomers, after an equilibration step of 15 minutes with 200 mM Na-acetate in 100 mM NaOH, 20µl of the sample was injected and a linear gradient to 700 mM Na-acetate in 100 mM NaOH within 40 minutes was started. Finally the column was washed for 5 minutes with 1 M Na-acetate in 100 mM NaOH and re-equilibrated for the next injection. For alginates oligomers, after an equilibration step of 15 minutes with 250 mM Na-acetate in 100 mM NaOH, 20µl of the sample was injected and a linear gradient to 1 M Na-acetate in 100 mM NaOH within 45 minutes was started. Finally the column was washed for 5 minutes with 1 M Na-acetate in 100 mM NaOH and re-equilibrated for the next injection.
4. Irradiation treatment and storage stability

Sealed tubes (Kimax) containing 2 mg sample and 2 ml of deoxygenated distilled water were irradiated with doses of 5, 10 and 15 kGy at a dose rate of 5.01 kGy/h. Irradiation using a Co-60 source was performed at the Institute for Atomic Sciences in Agriculture (ITAL) Wageningen, The Netherlands. Samples of control and irradiated polygalacturonic acid stored at -20°C for 3 and 6 weeks were analyzed to study their storage stability.

5. Statistic calculation

Analysis of variance for complete randomized design on average molecular weight, DP, and % hydrolysis of oligomers was calculated according to Steel and Torrie (1981).
IV. RESULTS AND DISCUSSION

A. Effect of Gamma Irradiation on Cell Wall Polysaccharides of Mango (*Mangifera indica* L.) var. Tommy Atkins

Solubility of cell wall polysaccharides from fruits does not occur only via action of endogenous enzymes but also physical process such as radiation using pasteurization dose.

The first experimental study on the effect of gamma irradiation on polysaccharides components, i.e., pectic substances, hemicellulose, and cellulose, was done using alcohol insoluble solids as starting materials. The technique was considered to be applied in this experiment as a simple preparation on isolation of the walls by washing in organic solvents. This isolation step extracts low-molecular weight sugars, amino acids, organic acids and many inorganic salts, and leaving behind an alcohol insoluble solids (AIS) containing polymers. These polymers will include intracellular proteins, RNA and starch, but the contaminants can often be ignored (Fry, 1988).
As a comparison, an analysis of pectin content derived from alcohol insoluble residue preparation of both unripe and ripe apple revealed that in unripe apple, mannose was undetected and the pectin contained more in rhamnose, arabinose, xylose, galactose, glucose and galacturonic acid residues. During ripening stage the neutral sugar composition of the extractable pectin did not change (Vries et al., 1981). Study on the effect of irradiation on AIS as measured from cellulose content, sugar composition (g/100 g pulp) and anhydrousuronic acid content resulted marginal differences between control and irradiated samples with regard to cellulose and anhydrousuronic acid content. No differences were found for rhamnose, arabinose, xylose, mannose, galactose, and glucose contents. The amounts of pectin soluble in alkali (ALKSP), hemicellulose and cellulose extractable from irradiated, at 10 kGy, and un irradiated AIS as dry powder or in suspended form (Figure 5). A comparative study on irradiated citrus fruit showed an agreement with the result that water and oxal solubil pectin showed an increased but protopectin prepared from NaOH fraction decreased in the irradiated materials. Methoxyl content and the activity of pectin esterase in peel decreased by
increasing radiation dose, and in membrane part irradiation
dose of 1.5 kGy could inhibit the enzyme activity but not at
the dose of 3 kGy (Rousse and Dennison, 1968). Effect of
gamma irradiation on pectic substances of alcohol insoluble
solids preparation from apple and carrot tissues under
storage condition have been investigated by other authors
(Mc.Ardle and Nehemias, 1956).

Figure 5. Effect of irradiation at 10 kGy on non-suspended and
suspended AIS as measured for ALKSP, hemicellulose and
cellulose from Na₂CyDTA extracted samples
This supporting result revealed that in all cases a breakdown of protopectin into pectate and soluble pectin has occurred. A decrease of the total pectic substances derived from 3 fractions, i.e., pectin, pectate and protopectin indicated a breakdown of pectin and pectate into simpler compounds as non pectic materials. The most extensive changes occurred in the protopectin constituent which was almost completely destroyed by the highest irradiation dose used in this experiment. Relative viscosities of the three pectic constituents of apple and carrots decreased. The destruction pattern in the pectic substances occurred simultaneously except in protopectin which was destroyed more rapidly. The percentage of Ca-pectate content derived from various pectin fractions showed that 77-90% of the alcohol precipitate were pectic substances.

Irradiation may have reduced the pectin contents (ALKSP) in the supernatants resulting from the mild alkali extraction. Hemicellulose content may have been increased by irradiation of suspended samples. Cellulose contents were reduced upon irradiation of dry as well as suspended AIS. Separate study on the changes in pectin substances due to enzymic activity
during ripening stage in mango Roe and Bruemmer (1981) showed the same result as in this experiment. They reported that a reduction in water and alkali soluble pectin related to loss of firmness indicated simultaneously by increasing the activity of endo-polygalacturonase and cellulase but a remarkable increase in ammonium oxalate soluble pectin was also found. The effect of irradiation on CyDTAIR fractions from non-suspended (powder) AIS is illustrated in Figure 6.

Figure 6. Effect of irradiation at 10 kGy on non-suspended AIS as measured for sugar composition and anhydro-uronic acid content in CyDTAIR fraction
There are no significant changes in sugar composition of the CyDTAIR fraction as a result of irradiation of dry AIS except for AUA which was absent after irradiation (Figure 6).

Powdered AIS (NS) and suspended AIS in water (S) either irradiated at 10 kGy or unirradiated AIS were extracted with Na₂CyDTA, and after centrifugation these supernatants were freeze dried, and the resulting powder of NS and S were examined for their sugar composition as well as their anhydrouronic acid content (Figure 7).

![Graph](image)

Figure 7. Effect of irradiation at 10 kGy on non-suspended and suspended AIS as measured for sugar composition and anhydrouronic acid content on Na₂CyDTASP
No significant differences were found between control and irradiated samples of NS and S AIS fractions as measured from Na$_2$CyDTA Insoluble Residue (CyDTAIR) (Figure 6), and dialyzed supernatant (Na$_2$CyDTASP) (Figure 7). It appears from Figure 7 that a remarkable result was obtained in unirradiated sample of suspended AIS. The anhydrouronic acid content is approx. 50% and this amount tends to increase after irradiation. Irradiation does not cause significant differences of the amounts of the various sugars in the Na$_2$CyDTA extracts of AIS irradiated in powder form or as suspension. The sugar contents of these pectin extracts (Na$_2$CyDTASP) are small, although contrary to the CyDTAIR fractions they contain arabinose and glucose.

A contrary result was found in the study on irradiated fresh strawberries at 4 kGy. Irradiation induces a degradation on galacturonic acid extracted from water insoluble pectin and from hemicellulose respectively. An extracted-irradiated cellulose resulted in increasing glucose content, meanwhile the neutral sugars originated from the pectin side chains were not influenced by irradiation (D'Amour et al., 1993).

Nevertheless some supporting results was also obtained from other authors. Voragen et al. (1983) reported that saccharides in
the ethanol insoluble residue of mango pulp had the highest of galacturonic acid and glucose; the arabinose was found high in pectin fraction and the saccharides in the hemicellulose fraction of the mango pulp contain more mannose that of the other fruits. Other comparative study as found by Kratchanova et al. (1991) revealed that the carbohydrate composition of pectins isolated from Guinean mango varieties by HCl extraction method at 85°C and pH 1.5 was estimated as: galacturonic acid (40-70%); arabinose (2-4%); Rhamnose (1-2%); xylose (1-7%); mannose (1-3%); galactose (14-22%) and glucose (8-22%). The same authors also reported that pectic substances extracted from dried Guinean mango pulp had polyuronide content of about 14.6-21.3%; molecular mass of the pectins was in the range of 72,000-83,000 Da, and carbohydrate composition were high in galacturonic acid, galactose and glucose, and low in arabinose, xylose, mannose and rhamnose. Doner (1985) demonstrated that a variety of neutral sugars, mainly rhamnose, galactose, arabinose, and xylose are present in pectin. This result is in agreement with this experiment as presented in Figure 7. Simpson et al. (1984) also demonstrated the occurrence of pectic substances such as pectin in several tropical fruits. It showed that in the unripe stage, pectin
embedded in a complex matrix with cellulose to form protopectin. In the fresh fruit, this pectin is protected by cellulose against pectolytic degrading enzymes or other natural constituents. The pectin content seems to increase from immature green stage to its peak value in the mature green stage, and then decreases during ripening. In this latest stage, protopectin decreases and pectin seems to be less protected by cellulose from activation of the pectolytic enzymes and finally resulting in increasing soluble pectin. Such degradation process is comparable to radiation treatment in certain condition.

ALKSP fractions were analyzed for the contents of neutral sugars and anhydrouronic acid. The sugar composition is not affected by irradiation, except for glucose and AUA. The amount of glucose and AUA are considerably decreased in the irradiated suspended sample. The anhydrouronic acid contents are nearly 50% decreased by irradiation in both (NS) and (S) fractions.

Results for the hemicellulose fractions obtained from irradiated and non irradiated AIS in powder form or suspended in water are compiled in Figure 3. As a result of irradiation the total xylose and glucose contents in the hemicellulose fractions are increased by nearly 50% as compared to the amount in the
non irradiated samples. Sum of the contents of xylose and glucose of the irradiated fractions of suspended AIS constitutes approx. 50% of the total amount of hemicellulose. This increasing amount of both sugars is probably xyloglucans are more easily extracted after irradiation of dry as well as suspended AIS. The contents of the other sugars and a hydrouronic acid are not significantly affected by irradiation for all fractions.

Figure 3. Effect of irradiation at 10 kGy on non-suspended and suspended AIS as measured for sugar composition and anhydrouronic acid content on hemicellulose fraction
Particularly in mango as finding by Voragen et al. (1983), the most important hemicelluloses are xylans, mannans or glucomannans and xyloglucans.

Fractionation of irradiated AIS, in dry as well as in suspended form resulted in small changes in the amount and composition of those fractions upon irradiation. Cellulose content were decreased in both NS and S of AIS. Degradation of cellulose by irradiation has also been reported by other investigator (Sreenath et al., 1987). The sugar composition of CyDTAIR did not change upon irradiation except for AUA which was virtually absent after irradiation treatment. Radiation-induced changes appear to result from several components. Therefore, it is advantageous to carry out experiments with polysaccharides model systems to elucidate specific features of degradation by irradiation.

The result on the elution behaviour of charge polysaccharides in the irradiation of soluble pectins as measured by HPSEC revealed that there is no shifting of the elution profiles into earlier retention time. This result might illustrate that there is
no changes on the distribution of methoxyl groups as also give an indication on negative reaction of a β-elimination.

Figure 9 presents the effect of irradiation on Na₂CyDTASP fraction of non-suspended AIS as measured from molecular weight distribution by HPSEC. The graph clearly demonstrates a reduction in the molecular weight distribution of the polysaccharides.

![Graph showing effect of irradiation on molecular weight distribution](image)

**Figure 9.** Effect of irradiation at 10 kGy on non-suspended AIS as measured for molecular weight in Na₂CyDTASP fraction
It can be explained that after irradiation at 10 kGy the main peak slightly shifts into longer retention time. It indicates that some degradation on the molecule chains occur in the large extent. A shoulder appears both in control and in irradiated sample may indicate a second population in the two samples having slightly different molecular weight. After irradiation at 10 kGy, it seems that shifting the main peak results in increasing the concentration of of lower molecular weight group as it is shown by an increase of shoulder peak. This situation may be already the case in the control where treatment may result in a shift to lower molecular weight for both populations. Variability in the range of molecular weight of each column of 3 HPGPC columns in series might contribute to the separations. The TSK 40XL column does not separate oligomers and only separate molecules between about 10-20,000 Dalton and 100,000 Dalton for pectins. TSK 30XL column separate mono and oligomers and it is able to separate molecules up to about 60,000 Dalton but the resolution is poor. The same result was obtained for the non suspended A18. Neutral sugars, AUA and cellulose content of A18 showed only marginal changes as a result of irradiation treatment.
B. Effect of Gamma Irradiation on Polysaccharides

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 was selected, which contrarily to pectins, is built up of different uronic and residues namely mannnuronic acid and guluronic acid in varying proportions and segments. Other starting material was prepared to mimic the methyl estergroups of pectin, also chemically modified propylene-glycol alginate was studied. As representative of neutral plant cell wall polysaccharides, arabinans with various degrees of branching were selected.

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) are presented in Table 1.

It can be seen that HMw pectin has higher values for avr. Mw and avr. Mn than LMw pectin, while alg. M/G 2.5 has lower value than in alg. M/G<1. Propylene glycol alginate type 109 seems to be more viscous than type 110.
Table 1. Characterization of pectin and alginates

<table>
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<tbody>
<tr>
<td>MHP</td>
<td>galact. acid</td>
<td>methyl esters</td>
<td>84000</td>
<td>62000</td>
</tr>
<tr>
<td></td>
<td>partially methylated</td>
<td>DM=76% with arabinose side chains</td>
<td></td>
<td></td>
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<tr>
<td>Na-Pectate</td>
<td>galacturonic acid</td>
<td>DM=2%</td>
<td>25000</td>
<td>12000</td>
</tr>
<tr>
<td>Alginates:</td>
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<td></td>
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<td>43100</td>
</tr>
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<td>M/G 2.5 and</td>
<td>mannuronic-guluronic acids</td>
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<td>69000</td>
<td>52000</td>
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<tr>
<td>M/G &lt;1</td>
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<td>73000</td>
</tr>
<tr>
<td>Type 110</td>
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<td></td>
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<td>37000</td>
</tr>
<tr>
<td>Highly-branched arabinans</td>
<td></td>
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</table>
Highly branched arabinans shows the highest value of Mw in comparison to the other selected polysaccharides.

The effect of irradiation of the selected polysaccharides was examined by measuring specific viscosity and also showed a decrease in their viscosity (Table 2). It can be seen that the specific viscosity decreased drastically (Appendix 1) by increasing radiation dose. This finding result might confirm that irradiation at doses up to 30 kGy induces glycosidic linkages in the solid samples, as indicated a reduction of viscosity. 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. D'amour et al. (1993) has reported that a hydrolysis process occurs in irradiated cellulose, cellulose derivatives as reported by Ebringerova et al. (1991), and for starches derived from different plant origin (Raffi et al., 1983).

Similar result on reducing viscosity induced by protopectin degradation was reported by Belli-Donini and Stronaiuolo (1969). The irradiated strawberries shows in reduction of total pectic substances and in viscosity; while a breakdown of HCl extractable protopectin was also demonstrated. Different
result was obtained in the study of enzymic hydrolysis of highly esterified pectin.

Table 2. The specific viscosity ($\eta_{\text{spec.}}$) of high methoxyl pectin and propylene glycol alginate irradiated in powder form at different relative humidity

<table>
<thead>
<tr>
<th>RH %</th>
<th>Irr. dose (kGy)</th>
<th>Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>HMP</td>
</tr>
<tr>
<td></td>
<td>$\eta_{\text{spec.}}$</td>
<td>$\eta_{\text{spec.}}$</td>
</tr>
<tr>
<td>0</td>
<td>0.30 ± 0.02</td>
<td>0.44 ± 0.10</td>
</tr>
<tr>
<td>40</td>
<td>15</td>
<td>0.17 ± 0.01</td>
</tr>
<tr>
<td>30</td>
<td>0.15 ± 0.02</td>
<td>0.18 ± 0.01</td>
</tr>
<tr>
<td>0</td>
<td>0.28 ± 0.02</td>
<td>0.41 ± 0.10</td>
</tr>
<tr>
<td>60</td>
<td>15</td>
<td>0.17 ± 0.01</td>
</tr>
<tr>
<td>30</td>
<td>0.13 ± 0.00</td>
<td>0.18 ± 0.02</td>
</tr>
</tbody>
</table>

Note: *) Average of 3 replications
**$t = $ flow time (second)
***$\eta = $ viscosity number

$$\eta^{**\text{spec.}} = \frac{[t^{**\text{sample}}] - [t^{**\text{water}}]}{(t^{**\text{water}})}$$
The pectin lyase endo-enzymes depolymerize the molecules at random, causing rapid drop in viscosity and only glycosidic linkages next to a methyl ester group are split by a β-eliminative mechanism (Pilnik and Rombouts, 1981). Other supporting results were demonstrated in irradiated apple pectin as reported by Sjoberg (1987), and for alginates (King, 1984). The results showed a reduction in molecular weight in which corresponds to the decrease in viscosity.

It can be seen from the same table that the specific viscosity of propylene glycol alginates decreased drastically by increasing radiation dose. This confirmed the finding result that gamma irradiation causes degradation of the backbone in the alginates. A relevant phenomena in reducing viscosity of alginates induced by radiation is confirmed by King (1994) who studied on irradiated Na-Alginate. The result shows a significant reduction in their molecular weight with corresponding changes in intrinsic viscosity, some changes in the functional properties and molecular weight of Na-alginate following irradiation was observed. Viscosity of Na-alginate in solution of 0.5 and 1% concentration decreased significantly with the increasing irradiation dose. The highest irradiation dose, 3.9 kGy, reduced
the gel strength about 30% and molecular weight reduced about three times in comparison to the untreated samples.

A general phenomena should be drawn from all the finding results that irradiation of Na-alginate at certain doses should be taken into account since the treatment resulting on the decrease of molecular weight as well as gel strength of the final products.

Other relevant result on the use of high energy gamma radiation subjected to 3 types of Na-alginate in powder form revealed that the irradiation dose of 50 kGy could reduce significantly their viscosity. These range of irradiation dose can only be applied to sterilize Na-alginate for absorbant and coagulant purposes in food industries, but it is not suitable for medical purposes neither as thickening nor gelling agent as reported by Kume and Takehisa (1983).

Since the viscosity of the charged polymers dropped drastically after irradiation, treated samples were also analysed by HPSEC. The calculated degrees of polymerization (DP) of pectins and propyleneglycol alginate (Prop-GlyAl 109) before and after irradiation, subsequent storage is summarized in Table 3. For both samples the degree of polymerization decreased with
increasing irradiation doses. Propylene Glycol Alginates with type 109 showed a larger degradation and also after irradiation. It is probably due to the differences in structural configuration which are responsible for this. Both in viscosity measurement and calculation of DP from those various samples were conducted under 2 different relative humidities. Degree of polymerization (DP) is calculated under following formula:

\[
DP = \text{weight of sample (g/ml)} \times \frac{1}{176}
\]

\[
\frac{\text{number of molecules}}{\text{weight of sample/avr. Mn}}
\]

These two humidities 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. (1992) revealed that high methylated pectin exhibit different behavior compared to low methoxyl pectin. At low relative humidity less than 60%, the sorbed moisture of high methylated pectin is very low, and the appearances of the materials is poor.
Table 3. Effect of irradiation on degree of polymerization (DP)* of high methoxyl pectin and propylene glycol alginate 109 as measured by HPSEC before and after storage at 23°C in powder form

<table>
<thead>
<tr>
<th>Sample</th>
<th>RH</th>
<th>Storage month</th>
<th>Degree of polymerization (DP) at irradiation doses of</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>0 kGy</td>
</tr>
<tr>
<td>HMP</td>
<td>40</td>
<td>0</td>
<td>330</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>210</td>
</tr>
<tr>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>60</td>
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<td>380</td>
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<td></td>
<td></td>
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<td>195</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>190</td>
</tr>
<tr>
<td>Prop- GlyAl 109</td>
<td>40</td>
<td>0</td>
<td>405</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>380</td>
</tr>
<tr>
<td></td>
<td></td>
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<td></td>
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<td>345</td>
</tr>
</tbody>
</table>

Note: *) Average of 3 replications
At higher relative humidity, at more than 60%, 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 availability 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 on carboxyl and methoxyl groups in the equilibrium moisture content is of important if a study on degradation mechanism of cell wall polysaccharides as well as pectin induced by irradiation will be elucidated. From the viscosity reduction and the decrease significantly in DP (Appendix 2) of irradiated samples as calculated from HPSEC
chromatogramme it is obvious that these type of polysaccharides are degraded by radiation up to 30 kGy.

It is not clear yet from these investigation that the depolimerization of such polysaccharides is a result of a hydrolytic process as described for cellulose (D'amour et al., 1993), cellulose derivatives (Ebringerova et al., 1991), and for starches derived from different plant origin (Raffi et al., 1983). Degradation of the backbone by other processes cannot be ruled out, while also rearrangement reactions of some chemical bonds next to the point of the cleavage reaction might take place possibly liberating new (reactive) fragments. The idea was that such products might react in the thiobarbituric acid assay, since this test is specially suitable for identification of malonaldehyde as radiolytic products in irradiated starches.

The results of the TBA test were negative for the pectin and alginate samples which were subjected towards irradiation with various doses. This excludes the formation of 4,5-unsaturated uronosyl residues as a product of cleavage of the backbone via 8-elimination (Rombouts, 1972). Subjection of samples of highly branched arabinan in solution to gamma
irradiation under nitrogen or oxygen then analyzed for the TBA test resulted that the irradiated arabinan showed a strong colour development. The maximum absorption was at 532 nm which had similar absorption wavelength to both malonaldehyde and 4,5-unsaturated galacturonic acid residue. It is difficult to explain such colour formation in TBA test since the complexity of radiolytical products and types of its radical-radical reactions present in irradiated highly branched arabinan is not identified in this experiment. Since arabinan are mainly built of sugar furanose units, the colour formation is also probably due to the unstability of their glycosidic linkages either at 1,3 or 1,5 position against irradiation, and forming new simpler compound which positively reacts with TBA. Absorption value increased with increasing radiation doses and was higher for irradiated arabinan in the presence of oxygen.

Figures 10 and 11 show the elution profiles of the two alginates at different alginates M/G ratio irradiated in powdered form respectively. It seems from Figure 10 that the irradiated alginates with M/G 2.5 at dose up to 30 kGy showed a shift of the elution pattern into longer retention time and the
shifting of each top peak after the treatment in comparison
to the unirradiated alginates. It is indicated that total
degradation has already occurred in the irradiated samples
before and after storage at direct effect reaction
predominant. This phenomena could be approved by the
following storage condition. Storage condition at 23°C for 2
months does not influence significantly shifting of elution
patterns particularly in irradiated samples. The same results
also occurs in irradiated alginate with M/G < 1 (Figure 11).
Separate calculation using GPC programme revealed that
higher in MM block had a degree of polymerization (DP) of
about 3-10 while for GG-block it was about 4-15. It is known
elsewhere that some physical properties of irradiated
alginites depend on the uronic acid composition and the
blockwise arrangement of acid residues. In this study M/G
2.5 has higher alternating sugar blocks which is more easily
degraded than M/G < 1. This result confirmed with the
statement of Pilnik and Rombouts (1985). This finding result
is building up a phenomena that the properties of irradiated
alginites is corresponding to alternate MG blocks to
determine the distribution pattern of those 2 acids and a
small percentage of alternating structures. Other
comparative studies have been done by some investigators. Structural characterization using liquid chromatography of alginates was carried out by Heyraud and Leonard (1990).

![Graph showing elution profiles of irradiated alginate M/G 2.5 before (I) and after (II) 2 months storage at 23°C as detected by HPSEC.](image)

**Figure 10.** Elution profiles of irradiated alginate M/G 2.5 before (I) and after (II) 2 months storage at 23°C as detected by HPSEC.
Figure 11. Elution profiles of irradiated alginates M/G <1 before (I) and after (II) 2 months storage at 23°C as detected by HPSEC.

Alginate types used were homopolymeric blocks of β-D-mannuronic acid (MM), α-L-guluronic acid (GG) and fraction
containing nearly the same portion of both monomers (MG blocks). They assumed that only GG blocks of a short length contribute to a stable junction formation with a few guluronic acid units. The gel strength is associated to the proportion of this sequence in the alginate chain.

The absence of β-elimination reaction in irradiated pectin is conducted using a commonly used titration method to examine the amount of ester groups in pectin. It was found that the values obtained did not show any significant difference for both the irradiated and untreated samples. This is in agreement with the above mentioned findings that no indications for a β-eliminative cleavage usually taking place next to an esterified uronosyl residue as observed by Kravtchenko (1992). Other experimental work is conducted to confirm the previous finding result using HPIEC method. This technique would reveal changes within the distribution of ester groups over the backbone, even when the absolute amount of esters remains constant. However, non-irradiated and irradiated HMP were found to elute in a number of comparable peaks at identical elution times as shown in Figure 12. Storage of HMP did not alter the elution profiles. So, it
should be concluded that there was no change in distribution of ester groups of irradiated HMP. These results are in agreement with Schols et al. (1989); and Deventer-Schriemer and Pilnik (1987). The suggestion that ester groups did not play a role in the mechanism of degradation was also confirmed by chromatographic separation of untreated and irradiated samples on basis of their charge density (Figure 12).

A contrary data was obtained from Sjoberg (1987). The result revealed that on irradiated certain types of polysaccharides as well as extracted pectin a significant effect on the reduction of degree of methylation was observed, and more degradation in pectic substances was found. Unfortunately, the reduction of degree of methylation does not occur in enzymic degradation of pectin molecules induced by glycosidases and lyases activities. Other supporting result on the effect of gamma irradiation on the molecular weight of isolated apple pectin was obtained from Clarke (1961). It is reported that immediately after irradiation, pectin was not depolymerized as indicated by the unchanged on molecular distribution.
Figure 12. Elution profiles of irradiated high methoxyl pectin as detected by HPIEC

Figure 13 illustrates irradiation with the doses of 15 and 30 kGy subjected to powdered-highly branched arabinan respectively did not alter their elution profiles as measured.
by HPSEC but the degradation of the arabinan both in unirradiated and irradiated samples are merely due to storage condition at 23°C for 2 months. The same figure shows significantly shifting of the elution profiles into longer retention time only occurred during storage. The radiolytic products as result of direct effect reaction predominant in the irradiated arabinan seems to be less reactive in solid state. However, there is a possibility that free radicals might react with the highly branched molecules side chains as favourable radical attack. The stability of highly branched arabinan against irradiation in solid state is influenced by the presence of radical scavengers, since the purity of highly branched arabinan is doubtful. The isolation step of arabinan from the plant origin containing sugars have some difficulties in particular when the arabinans attach to the pectin molecules.
Figure 13. Elution profiles of irradiated highly branched arabinan in powdered form before (I) and after (II) 2 months storage at 23°C as detected by HPSEC.
Other types of arabinans, linear arabinan, was dissolved in deoxygenated distilled water and irradiated with doses 5 and 15 kGy respectively then measured by HPAEC (Figure 14).

The linear arabinan type 50 showed a higher degree of polymerization than the other types but irradiation with 5 kGy

Figure 14. Elution profiles of irradiated linear arabinan type 50 in solution as detected by HPAEC
caused a similar shift of the retention time to lower values indicating strong depolymerization. It can be explain from the result that degradation of the irradiated simple polymer in solution using low concentration an indirect effect reaction via the solvent is predominant. The differences in the degradation modes of other types arabinan are due to the concentration and the arrangement of the molecules among the main chain.

C. Degradation of Fragmented Polysaccharides Induced by Irradiation

Isolation and characterization of the low molecular weight carbohydrate fragments were the main strategy using oligosaccharides to study the effect of irradiation and to reveal the mechanism of degradation. Oligomers of appropriate length were isolated since they were not commercially available.

Figure 15 illustrates the degradation of polygalacturonic acid (PGA) by endopolygalacturonase (PG) as monitored by HPSEC.
Broad shoulders appear in the treated samples indicate a second population present in the samples which has slight differences in molecular weight.

Using commercial GPC software, it was calculated that the average DP of PGA digested for 2.5 hours at 30°C was ca.10, which was in the range of analysis by HPAEC. However, from the same figure, it can be seen that the digest still had a rather broad Mw distribution. Therefore it was considered that a further chromatographic separation over Sephacryl S100 might be useful.
Figure 15. The degradation of polygalacturonic acid (PGA) by endo polygalacturonase (endo-PG) as detected by HPSEC.
The separation obtained on Sephacryl S100 was very similar to that obtained by HPSEC and the fraction were pooled into 3 subfractions, i.e., HMw, MMw and LMw and analyzed again by HPSEC (Figure 16). It appear in this figure that fractionation of degraded polymer by Sephacryl S100 chromatography resulting in LMw fragment (fraction c) contains separated smaller oligomers (Mw about 200 = monomer and ca. 2000 = ca. decamer). The irregular peaks appear in the chromatogram is due to the different separation ability through 3 different molecular weight range of HPGPC columns in series. These column is set in series (TSK 40XL, 30XL, and 20XL) might contribute to the separated material. TSK 40XL does not separate oligomers but only separate molecules having molecular weight is about 10,000-20,000 Dalton and 100,000 Dalton for pectin. While TSK 30XL column is able to separate monomer, oligomer, and molecules < 60,000 Dalton. TSK 20XL column separates monomer, trimer and pentamer quite well but is unable to separate molecules having a mass higher than 10,000 Dalton.
Figure 16. Fractionation of polygalacturonic acid digest into HMw, MMw, and LMw subfractions using Sephacryl S100 detected by HPSEC.
The characteristics of the pools before and after irradiation with respect to their avr. Mw, avr. Mn, Mw/Mn and DP values determination, is shown in Table 4. Percentage of hydrolysis can be calculated as:

\[ \Delta \text{number of molecule}^* \]

\[ \% \text{hydrolysis} = \frac{\Delta \text{number of molecule}}{\text{weight of sample}} \times 100 \]

\[ \frac{176}{100} \]

\[ \Delta \text{number of molecule}^* = \Delta \text{number of molecule after irradiation and number of molecule before irradiation.} \]

It can be seen from this table that the Mw/Mn ratio is close to 1, indicating that the fractions are rather homogenous, although especially for the MMw subfraction, and distinct peaks for the individual oligomers. It can be summarized from Table 4 that the effect of degradation depend on the size of the oligomers under investigation since 6.7% of all bonds present in the HMw subfraction were hydrolysed (5 kGy dose), while the degradation for the MMw and LMw subfractions were only 1.8 and 0.8% respectively. These values were almost doubled when a irradiation dose of 10 kGy was used, while the extra effect of the 15 kGy dose was less pronounced.
Table 4. Weight average molecular weight (avr. Mw), average number molecular weight (avr. Mn), degree of polymerization (DP), and hydrolysis (%) of irradiated HMw, MMw, and LMw sub fractions of PGA digest as measured by HPSEC

<table>
<thead>
<tr>
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<tr>
<td>HMw</td>
<td>0</td>
<td>9600</td>
<td>8100</td>
<td>1.2</td>
<td>40.4</td>
<td>0</td>
</tr>
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<td>4100</td>
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<td>1.9</td>
<td>10.9</td>
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<td>1200</td>
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<td>6.1</td>
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</tr>
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<td>2700</td>
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<td>13.4</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>5</td>
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<td>2150</td>
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</tr>
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<td>1900</td>
<td>1.4</td>
<td>9.3</td>
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</tr>
<tr>
<td></td>
<td>15</td>
<td>2400</td>
<td>1600</td>
<td>1.5</td>
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<tr>
<td>LMw</td>
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<td>4.2</td>
<td>0</td>
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<td></td>
<td>5</td>
<td>1000</td>
<td>810</td>
<td>1.2</td>
<td>4.1</td>
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<td>730</td>
<td>1.3</td>
<td>3.6</td>
<td>3.7</td>
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</table>

The following result can be recognised in the elution profiles of the irradiated PGA MMw subfraction as monitored by HPSEC (Figure 17).
Figure 17. Elution profiles of MMw polygalacturonic acid subtraction before and after irradiation as detected by HPSEC.
It can be explained from Figure 17 that since only some of the molecules are degraded by radiation, at 5 kGy, the peak top will remain at the same position as in unirradiated sample. The differences among the population as degradation products in the treated sample might elute later and resulting broader peak. The broader peak is known as hydrodynamic volume. Hydrodynamic volume can be explained as volume which is occupied by a molecule in the solution. The charge molecule will give repulsion, and showing greater hydrodynamic volume. When the molecule is linear, the volume is greater in comparison to a highly branched molecule at the same molecular weight. It can be suggested that it is necessary to calibrate size exclusion chromatography (SEC) column to have better explanation about hydrodynamic volume as well as in HPSEC elution profiles by calibrating similar type of polymer. When the irradiated sample degrades to lower oligomers, the retention of the main peak will be completely shifting into longer time. The calculated DP values of 40, 13 and 4 for HMw, MMw and LMw PGA subfractions respectively (Table 4). HMw is representing fragment which the degradation process following radiation could be monitored by HPAEC.
It can also be seen from Figure 17 that the molecular weight decreased significantly during irradiation and the shift in Mw tends to increase by increasing radiation dose.

For the MMw subfraction as well as for the two other fractions, the effect of three different irradiation doses is presented in Table 5. Radiation treatment with doses up to 15 kGy at room temperature could degrade significantly (Appendix 4) 3 fractions of PGA digest, and resulting a reduce in degree of polymerization. Unlike enzymic degradation of commercially extracted pectin and citrus using pectin esterases. It is reported that the molecules are deesterified by that enzymes without affecting their degree of polymerization (Filnik and Rombouts, 1981). Since it has been known that irradiation might induce chemical changes in the polymer molecules as result either from the direct-effect or indirect-effect reactions (Charlesby, 1960), the irradiated samples were stored at -20°C for several weeks and analyzed at certain intervals. The data revealed that the irradiated samples did not show any continuation of the degradation process during storage at low temperatures. The small variation in DP values found for the various fractions during storage are within
experimental errors. The storage stability found is not in contrast with findings of Nawar (1983) who stated that irradiated molecules may continue to produce excited and ionized molecules which immediately begin to react with each other to form some new stable chemical radiation products. It might also be expected that during storage at temperatures below zero the reactivity of radiolytic products will be diminished due to protection by ice. The intermediate products are generated through water radiolysis via indirect effect reaction predominant. These species are trapped in frozen materials, and unable to react each other or with the substrate. In this situation freezing condition creates strong protective effect on the reactivity of free radicals.
Table 5. Effect of irradiation and storage at -20°C on degree of polymerization (DP) of HMw, MMw, and LMw subfractions of PGA digest as measured by HPSEC

<table>
<thead>
<tr>
<th>Storage time week</th>
<th>Irr. dose kGy</th>
<th>Subfraction [s]</th>
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<tr>
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<tr>
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</tr>
<tr>
<td>15</td>
<td>6.6</td>
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Figure 18 illustrates typical elution profiles of non-treated and treated samples of galacturonic acid oligomers (MMw subfraction) as measured by HPAEC. It should be realized that in contrast to HPSEC with refractive index detection,
examination of the HPAEC elution profiles is not an appropriate method for absolute quantification of oligomers formed, because the response factor of the PAD detector for the oligomers might be different (Lee, 1990). For this reason, the contribution of the higher oligomers in the digest is underestimated.

Figure 18. Elution profiles of MMw polygalacturonic acid subfraction as detected by HPAEC
As a result of irradiation, the major peaks in the elution profiles (galacturonic acid oligomers of DP 5-11) are degraded and peaks eluting at lower salt concentrations increased in intensity. Using standards available in the laboratory, it was demonstrated that these oligomers formed were "normal" oligogalacturonic acid oligomers having a lower DP and were the result of a hydrolytic cleavage of the glycosidic bonds. The results suggest that using irradiation, galacturonic acid oligomers (DP > 8) were preferentially degraded in a random fashion, comparable to the mechanism as described for polygalacturonase by Piñik and Rombouts (1981).

These findings are also in agreement with those of Skinner and Kertez (1960), who stated on basis of viscosity measurements and electrophoreses experiments that a hydrolytic fissure of the glycosidic linkages is expected. Increasing irradiation doses induces more degradation in pectin molecules are mostly found in this experimental work rather than cross linkages. However, especially irradiation at high irradiation doses resulted also in the formation of some unknown compounds as intermediate products eluting in between the known galacturonic acid oligomers, suggesting a
more complex mechanism then hydrolysis of the glycosidic linkages only. Although the amount of these unknown compounds is much lower than the amount of the "normal" saturated oligogalacturonic acids, these compounds might be crucial intermediates in the elucidation of the degradation pathway. Since the above findings demonstrated that the selected methods enables a more detailed examination of irradiated oligo-galacturonides, also oligosaccharides derived from other charged polymers were studied using the same approach.

Pectin and alginate samples were mechanically degraded by a mechanolysis method and resulted in fragments with unchanged degree esterification was also found in the study of Deventer-Schriemer and Pilnik (1987). The molecular weight monitored by HPSEC of high methoxyz pectin and alginites decreased significantly within 55 h and 84 h milling respectively and the average degree of polymerization obtained was about 20. The degraded polymers were separated on Sephacryl S100 as described above and the fraction obtained were pooled into the subfractions: HMw, MMw and LMw. These three subfractions of HMP and the alginites were
then characterized with respect to their molecular weight as measured by HPSEC. DP values of 28; 22, and 10 were calculated for the pectin samples respectively. The corresponding values for the alginate having a M/G ratio of 2.5 were 38; 20, and 7 and for the alginates with a M/G ratio<1 were 25; 22, and 8 respectively.

Table 6 presents the results of irradiation induced degradation on the subfractions derived from the various degraded pectin and alginates samples. It is found that for all subfractions, the average DP decreased with increasing irradiation dose. This is in agreement with results obtained by King (1994), and Kume and Takehisa (1983) for alginate polymers, which showed in reduction in viscosity and reduction in gel strength upon molecular degradation induced by radiation for high viscosity Na-alginates. The effect of irradiation towards oligomers of pectin is rather similar to HPSEC elution profiles (not shown), decrease in average DP (Table 6), and average Mw and Mn (not shown) as compared to the effect measured for oligomers of polygalacturonic acid (Figure 19).
Table 6. Effect of irradiation on degree of polymerization (DP) of pectin, alginate M/G 2.5 and alginate M/G <1 fragments as measured by HPSEC

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<td>HMw</td>
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</tbody>
</table>

Unfortunately, the degree of esterification was not measured. However, various authors showed that radiation did not affect the degree of esterification of pectin (Sjoberg, 1987). Comparison of the results obtained for alginites showed that alginites are more sensitive to radiation than pectin. It can be seen in the HMw subfraction that an alginate having a M/G ratio of 2.5 seems to be more sensitive than an alginate having
relatively more guluronic acid residues ($M/G < 1$; Table 6). Also in studies of heat induced degradation, it was found that alginates with a high $M/G$ ratio were more susceptible to degradation. From this it appears that the uronic acid composition and arrangements of the uronic acid residues (blockwise, alternating) affects the stability of the alginates. The degradation of pectin and alginates fractions by irradiation was also measured by HPAEC (Figures 19 and 20). In the elution profiles of the irradiated pectin fragments, the most pronounced degradation products could be identified as galacturonic acid oligomers. However, due to the HPAEC elution conditions, methyl esters which still might be present linked to the carbonyl groups of galacturonic acid residues, will be saponified during the analysis and no information will be obtained on the esterification after irradiation. Comparison to the elution profiles of irradiated pectin oligomers (Figure 19) with that of irradiated PGA oligomers, illustrates that the mechanism of degradation of uronic acid oligomers as provoked by irradiation is not influenced significantly by methyl esterification. On the other hand, it is assumed that degradation after irradiation is not stimulated by negatively charge carboxyl groups. Nevertheless, it should be stated that
small differences between HPAEC profiles of the methyl esterified and the non-methyl esterified galacturonic acid oligomers can be recognized, especially within the ratio of minor, intermediate products indicated as unknown peaks towards the more dominant galacturonic acid oligomers formed after treatment.

Figure 19. Elution profiles of MMw pectin subfraction as detected by HPAEC
The elution profiles of irradiated alginates are much more complicated (Figure 20) since "simple" hydrolysis of the glycosidic linkages (as found for galacturonic acid oligomers) may result in numerous oligomers having the same size, but differing in building blocks (guluronic acid versus mannuronic acid) or sequences of these residues.

Figure 20. Elution profiles of MMw alginate M/G 2.5 subfraction as detected by HPAEC
Consequently, PGA digest is only applicable as standard to indicate radiation induced degradation of pectin. The elution profiles of the two different irradiated alginates oligomers (M/G =2.5 versus M/G<1) showed great similarities, although the chromatogram of the alginate having a M/G ratio of 2.5 showed more unknown peaks. The degradation can be ascribed to radiation induced fracture of the main chain.

A comparative study on the effect of gamma irradiation on the structure of semi purified citrus pectin as a model system was conducted. Massey (1968) analyzed the treated samples by electrophoresis, and he concluded that irradiation create random cleavage of glycosidic linkages along its length yielding fragments with lower molecular weight of pectin molecules. The heterogeneity of pectin fragments distribution increase in wide range at the dose above 2 kGy. It is also reported that changes such as deesterification which would alter the charge of pectin molecules did not occur. A confirmation result is also obtained by other author who studied on the effect of γ irradiation on the digestibility of the pectin polymers. It reveals that a hydrolysis process in the
macromolecules in the cell-wall took place to form simpler compounds (Murray, 1990).

d. General Discussion

It is well understood that the presence of some radical scavengers as proton donor such as vitamin and protein in the fresh fruit might create a complex matrix. This situation create some difficulties in order to elucidate a degradation mechanism as well as immediate softening in the fresh fruit following radiation. Such mechanism can be studied using irradiation of selected cell wall polysaccharides as model systems in various conditions.

Effect of gamma irradiation at dose of 10 kGy on the isolated fruit cell wall represented as alcohol insoluble solids has been conducted. Remarkable decrease in pectin, and cellulose contents both in non-suspended and suspended AIS samples after irradiation were shown. The mechanism proposed for irradiation of carbohydrate in solid state (Figure 21), and cellulose degradation pathway based upon cellubiose as cellulose derivatives are presented in Figure 22 and Figure 23
respectively as in agreement with Sonntag (1980). Some neutral sugars such as xyloglucans show an increase in irradiation of suspended AIS in CyDTAIR, Na₂CyDTASP, and hemicellulose fractions respectively. Xyloglucan is hemicellulose fraction which absorbs to the cellulose fibrils and as cementing agent between those fibrils. A gamma irradiation induces degradation on xyloglucan and resulting losses of cementing function which directly reflects to the texture. A reduction in molecular weight is found in irradiated Na₂CyDTASP fraction, while no changes in charge density of the irradiated pectin is observed.

\[
\text{Molecule (M)} \quad \xrightarrow{\text{e}^-} \quad \text{M}^+ + \text{e}^- \\
\text{M}^+ + \text{M} \quad \xrightarrow{\text{e}^-} \quad \text{N}^+ + \text{P} \\
\text{M}^+ + \text{e}^- \quad \xrightarrow{\text{e}^-} \quad \text{M}^* \rightarrow \text{radicals} \\
\text{N}^+ + \text{e}^- \quad \xrightarrow{\text{e}^-} \quad \text{N}^* \rightarrow \text{radicals}
\]

Figure 21. Radical formation induced by ionizing radiation of solid state or direct effect reaction predominant (Sonntag, 1980).
These results give an idea that irradiation at the dose of 10 kGy could degrade some cell wall components into smaller entities. However, these finding data would not be sufficient to to elucidate a degradation mechanism in irradiated fruit induced softening.

Figure 22. Proposed mechanism for the radical-induced scission of the glycosidic linkage at C4 or C1 of cellulose derivatives
Figure 23. Proposed mechanism for the radical-induced scission of the glycosidic linkage at C5 of cellulose derivatives.
It is probably due to the less reactive radicals, and to the complexity of isolated cell wall polysaccharides of AIS as starting materials, which contains some unreleased contaminants such as intra cellular proteins, RNA and starch. These contaminants might react in the molecules as radical scavengers during irradiation. Based upon these reasons, it would be wise if further experimental work on polysaccharides model systems could also be carried out.

A study on the effect of gamma irradiation at medium and high doses on polysaccharides model systems is reported. The radical formation induced by ionizing radiation of solid state materials has been illustrated by Sonntag (1980) (Figure 21).

Polysaccharides model systems which consist of uronic acid residues of various sugars building blocks were selected as starting materials. There is similarity in the result as in the previous work that irradiation could degrade pectin molecule, and also alginates into smaller entities via hydrolysis. This result is estimated from the reduction in viscosity, shifting of high molecules into lower molecular weight, and a decrease in the degree of polymerization. It is again, demonstrated that
charge density of high methoxylated pectin remains constant after irradiation.

Such phenomena can already be formulated from those two research works that radiation attacks cell wall components and the model systems in random, and hydrolyzes glycosidic bonds (Figure 24) but does not create splitting of high methoxyl pectin backbone through $\beta$-elimination reaction due to stability of proton at $\beta$-carbon. It seems that irradiation is not easy to remove proton of high methoxyl pectin. The proton will be easily removed when the electron density /free electron around carbon is high (Figure 25).

![Diagram of pectin structure](image)

Figure 24. Proposed mechanism for irradiation of high methoxyl pectin at direct effect reaction predominant
However, it is considered necessary to conduct other research on simpler polysaccharides model systems viz. as oligomers if further valuable information is still needed.

Figure 25. Proposed mechanism for the negative β-elimination reaction on irradiation of high methoxyl pectin in solid state
Irradiation of various oligomers in solution at doses of 5 - 15 kGy has been observed. These oligomers at certain DP were obtained by enzymic degradation for polygalacturonic acid, and by mechanalysis for pectin, and alginates then classified into 3 different molecular weight. Water radiolysis will generate some reactive free radicals as radiolytic primary products which are ready to attack solute materials as indirect effect reaction (Figure 26). The solute materials are degraded into larger extent and resulting some new fragments with lower in molecular weight (Figure 27).

\[
\text{rays} \quad \overset{\text{H}_2\text{O}}{\longrightarrow} \quad \text{OH}, \text{H}, \text{e}^-\text{aq}, \text{H}_2, \text{H}_2\text{O}_2, \text{H}^+, \text{OH}^-
\]

\[
\text{OH}^- \text{ or H}^+ + \text{H} \rightarrow \text{OH} \quad \text{H}_2\text{O} (\text{H}_2)\text{O} \quad \longrightarrow \quad \text{OH}
\]

\text{solute carbohydrate}

\[
\text{in oxygenated solutions, the primary radicals are scavenged by molecular oxygen}
\]

Figure 26. Proposed mechanism for irradiation of solute carbohydrate in the presence of oxygen
The presence of intermediate products as unknown peaks in between monomer and dimer, etc in irradiation of oligomers on solute state might give an indication that radiation does severe random attack on the oligomer samples.

It is obvious from the whole results that irradiation of uronic acid residues from various types of sugar building blocks either in isolated cell wall, selected polysaccharides model systems, or in oligomers is more likely degradation rather than cross linking. Radiation treatment of cell wall polysaccharides both in solid state and in solute somewhat show certain similarity in the degradation process as occurring in the enzymatic liquefaction of plant cell wall or in chemical treatments.

The primary products formation

$H^\cdot; OH^\cdot$ (the most reactive species);

$H_3O^\cdot; e_{aq}^-; HO_2^\cdot; H_2O_2$.

![Diagram of polygalacturonic acid molecule]

attacked by:

$H^\cdot; OH^\cdot$, etc.

Figure 27. Proposed mechanism for possible radicals attack on galacturonic acid irradiated in solute state
V. CONCLUSION

It is obvious from the finding results that gamma irradiation induces degradation on isolated cell wall components of mango fruit, i.e., pectin, hemicellulose, cellulose and the model systems either in solid or in solute state. The conversion of insoluble wall-bound protopectin of high molecular weight to water-soluble pectin was remarkable by reducing a number of molecular weight, and the decrease in specific viscosity. Some losses of pectin from cell wall were consistent with observed decreases in cell wall galactose, galacturonic acid, and highly branched arabinan, and increases some simpler sugars. Presence of water and oxygen during irradiation of samples might accelerate the degradation process. Immediate softening of fruits after irradiation, which is considered as an adverse effect, can be correlated with the degradation of the wall components in solute state. Such evaluation can only be approached using cell wall model systems in order to elucidate the degradation mechanism and their chemical process occur in the complex polymer after irradiation.
Effect of gamma irradiation of a dose at 10 kGy on isolated cell wall polysaccharides of mango by alcohol precipitation as starting material showed marginal changes on neutral sugars, except in xyloglucan, anhydrousuronic acid, and cellulose content. The sugar composition of the insoluble residue after extraction with Na_2CyDTA did not change upon irradiation except for anhydrousuronic acid which was virtually absent after irradiation treatment.

Irradiated pectins, alginates and arabinans with doses of 1 to 30 kGy stored at various relative humidities resulted in reduction in molecular weight and specific viscosity following irradiation may be due to the effect of free radicals on splitting the glycosidic bonds. The degradative effect increased with increasing irradiation dose. Some differences were also found when polymers were irradiated in solutes, and in solid state. Degree of esterification remained constant in irradiated pectin in comparison to the untreated pectin. The chromatographic separation was done on the basis of charge densities of ester groups.

Fragmentation of polysaccharides to oligomers within a molecular weight range which enables chromatographic
characterization of the fragments provided good model substrates to study their degradability under irradiation conditions, and to monitor the products formed by HPSEC and HPAEC methods. The low molecular weight fragments of PGA, pectin, and alginates exposed to radiation, show a reduction in weight average of molecular weight. Degradation by irradiation can be ascribed to hydrolytic cleavage of glycosidic bonds resulting in new fragments with lower degree of polymerization and formation of other reaction products considered as intermediate species appearing on chromatograms between known peaks which could not be identified by methods used in this study.

A general phenomena can be postulated that degradation mechanism on gamma irradiation of cell wall polysaccharides as well as in model systems seems to be random cleavage and hydrolysis pathways. There is negative indication either on the double bond formation or β- elimination reaction on high methoxyl pectin irradiated in solid state. Irradiation splits oligomers in solute state at the glycosidic linkages next to free carboxyl groups, and liberating some new fragments of intermediate radiolytic products.
VI. RECOMMENDATION

Cell wall polysaccharides model systems were selected in this research in order to elucidate a degradation mechanism of immediate softening occurs in irradiated fresh fruits.

Gamma irradiation is safe, effective and efficient techniques to preserve fresh fruits but irradiation could degrade the wall components and resulting in textural loss. Ionization process following radiation in irradiation of cell wall polysaccharides creates some free radical which are readily attack on the molecules of the wall components. The rate of degradation pathways is in random depending on the concentration of the solute materials; hydrolysis occurred on the glycosidic linkages, and producing some new components with lower molecular weight indicated by increasing soluble materials and reducing the specific viscosity.

Treatment of fruits with ionizing radiation can produce a wide variety of beneficial effects such as the extension of shelf-life; inactivation of insects, parasites, mould and yeasts; and the delay of ripening. Underlying from the finding results in this study revealed that irradiation of fresh fruits as single treatment
is not recommended. Moreover, mature green stage of climacteric fruits seems to be an optimum condition prior to irradiation exposure is due to the balance of moisture and the stability of the cell wall components in the fruit against irradiation.

However, further application for the results of model systems into real mango needs additional consideration since mango is a complex system. The same chemical products are formed in food are also formed in dilute solutions or in simple component substances. There are often however, large quantitative differences.

The radiolytic effect in fresh fruit can be minimized by irradiating the fruit in combination treatments such as modified and control atmosphere, dipping and coating. It is also necessary to irradiate the fruit at high dose rates, and using radical scavengers as protective agents against depolymerization of cell wall components.

The effect of radical-radical reaction generated from gamma irradiation on cell wall components still open question since the mechanism rather complicated. It is important to do further research work on identification and to quantify the unknown compounds as intermediate radiolytic products which come along in between monomer and dimer peaks, dimer and
trimer peaks, etc. Qualitative and quantitative analysis on type of monomer, dimer, trimer of oligomers after irradiation should also be identified.
VII. REFERENCES


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**Raffi** 


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Urbain, W.M. 1989. Food Irradiation: the past fifty years as prologue to tomorrow, Recent government actions affirming the safety of irradiation should encourage the food industry to proceed with its utilization. J. of food Technology July: 78 and 92.


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### Appendix 1. Analysis of variance on the specific viscosity (η spec.) of high methoxyl pectin and propylene glycol alginate irradiated in powder form at different relative humidity

<table>
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<th>MS</th>
<th>F-calc.</th>
<th>F-test</th>
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<td></td>
<td></td>
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<td>1 %</td>
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<td>0.0527165</td>
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<td>0.0020633</td>
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<td>Total</td>
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<td>0.441283</td>
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<td></td>
<td></td>
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</tbody>
</table>

** = highly significant at p>0.01  
* = significant  at p>0.05
Appendix 2. Analysis of variance on effect of irradiation on degree of polymerization (DP) of high methoxyl pectin and propylene glycol alginate before and after storage at 23°C in powder form

<table>
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<tr>
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<th>F-test</th>
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<td></td>
<td></td>
<td>5 %</td>
</tr>
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<td>1373.4**</td>
<td>3.89</td>
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<td>1030.9**</td>
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** = highly significant at p>0.01  
* = significant at p>0.05
Appendix 3. Analysis of variance on weight average molecular weight (Avr. Mw), number average molecular weight (avr. Mn), degree of polymerization (DP), and hydrolysis (%) of irradiated HMw, MMw, and LMw subfractions of PGA digest as measured by HPSEC

<table>
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<th>F-calc.</th>
<th>5 %</th>
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</tr>
<tr>
<td>% Hydr.</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Sub-fact.</td>
<td>2</td>
<td>364.807</td>
<td>182.403</td>
<td>1124.40**</td>
<td>4.41</td>
<td>8.28</td>
</tr>
<tr>
<td>Irrad.</td>
<td>2</td>
<td>90.726</td>
<td>45.363</td>
<td>279.63**</td>
<td>4.41</td>
<td>8.28</td>
</tr>
<tr>
<td>Interact.</td>
<td>4</td>
<td>19.893</td>
<td>4.973</td>
<td>30.60**</td>
<td>2.93</td>
<td>4.58</td>
</tr>
<tr>
<td>Error</td>
<td>18</td>
<td>2.920</td>
<td>0.1622</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>26</td>
<td>478.3467</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

** = highly significant at p > 0.01
* = significant at p > 0.05
Appendix 4. Analysis of variance on effect of irradiation and storage at 23°C on degree of polymerization (DP) of HMw, MMw, and LMw subfractions of PGA digest as measured by HPSEC

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>SS</th>
<th>MS</th>
<th>F-calc.</th>
<th>F-test 5%</th>
<th>F-test 1%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Storage(S)</td>
<td>2</td>
<td>0.9217</td>
<td>0.46085</td>
<td>1.51</td>
<td>3.13</td>
<td>4.92</td>
</tr>
<tr>
<td>Irrad. (I)</td>
<td>3</td>
<td>3518.70</td>
<td>172.90</td>
<td>3831.0**</td>
<td>2.74</td>
<td>4.08</td>
</tr>
<tr>
<td>Subfr. (F)</td>
<td>2</td>
<td>2786.75</td>
<td>1393.3</td>
<td>4552.3**</td>
<td>3.13</td>
<td>4.92</td>
</tr>
<tr>
<td>Interact.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S/I</td>
<td>6</td>
<td>7.045</td>
<td>1.174</td>
<td>3.84**</td>
<td>2.23</td>
<td>3.07</td>
</tr>
<tr>
<td>S/F</td>
<td>4</td>
<td>15.233</td>
<td>3.808</td>
<td>12.44**</td>
<td>2.50</td>
<td>3.60</td>
</tr>
<tr>
<td>I/F</td>
<td>6</td>
<td>4485.98</td>
<td>747.66</td>
<td>2442.6**</td>
<td>2.23</td>
<td>3.07</td>
</tr>
<tr>
<td>S/I/F</td>
<td>12</td>
<td>9.242</td>
<td>0.7702</td>
<td>2.516**</td>
<td>1.89</td>
<td>2.45</td>
</tr>
<tr>
<td>Error</td>
<td>72</td>
<td>22.038</td>
<td>0.30608</td>
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<tr>
<td>Total</td>
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<td>3268.437</td>
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</tr>
</tbody>
</table>

** = highly significant at p > 0.01
* = significant at p > 0.05
Appendix 5. Analysis of variance on effect of irradiation on degree of polymerization (DP) of pectin, alginate M/G 2.5, and alginate M/G<1 fragments as measured by HPSEC

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>SS</th>
<th>MS</th>
<th>F-calc.</th>
<th>F-test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>5 %</td>
</tr>
<tr>
<td>Sample (S)</td>
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<td>305.0067</td>
<td>152.503</td>
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<td>3.13</td>
</tr>
<tr>
<td>Subtract. (F)</td>
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</tr>
<tr>
<td>Irrad. (I)</td>
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<td>2865.6167</td>
<td>955.205</td>
<td>1316.01**</td>
<td>2.74</td>
</tr>
<tr>
<td>Interact.</td>
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<td></td>
</tr>
<tr>
<td>S/F</td>
<td>4</td>
<td>130.1833</td>
<td>32.546</td>
<td>44.83**</td>
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<tr>
<td>S/I</td>
<td>6</td>
<td>174.0133</td>
<td>39.002</td>
<td>39.95**</td>
<td>2.23</td>
</tr>
<tr>
<td>F/I</td>
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<td>1336.9133</td>
<td>222.819</td>
<td>306.98**</td>
<td>2.23</td>
</tr>
<tr>
<td>S/F/I</td>
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<td>30.533</td>
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<td>1.89</td>
</tr>
<tr>
<td>Error</td>
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<td>52.2600</td>
<td>0.7258</td>
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<tr>
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<td>107</td>
<td>6482.8967</td>
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</tbody>
</table>

** = highly significant at p>0.01
* = significant at p>0.05