PENGARUH IRADIASI GAMMA PADA HASIL ISOLASI DINDING SEL DAGING BUAH MANGGA *(Mangifera indica L.)* var.Tommy Atkins.

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ABSTRAK

PENGARUH IRADIASI GAMMA PADA HASIL ISOLASI DINDING SEL DAGING BUAH MANGGA (*Mangifera indica* L.) var. Tommy Atkins. Iradiasi merupakan salah satu metode pengawetan yang dapat dugunakan untuk memperpanjang masa simpan produk pertanian dengan cara menekan pertumbuhan serangga, dan menurunkan pertumbuhan mikroba secara efektif. Iradiasi pada buah segar pada dosis pasteurisasi dapat menimbulkan pengaruh pada tekstur buah seperti hilangnya integritas dari komponen dinding sel buah. Komponen utama dinding sel polisakarida terdiri dari substansi pektin, hemiselulosa dan selulosa. Ketiga jenis komponen tersebut memiliki peranan penting di dalam proses pelunakan yang terjadi seketika pada buah akibat radiasi. Mekanisme pelunakan karena proses degradasi dapat diketahui dengan mempelajari hasil pemecahan komponen dinding buah akibat proses tersebut. Dinding sel polisakarida yang bersifat tidak larut dalam alkohol selanjutnya dipisahkan dari daging buah mangga digunakan sebagai sampel yang akan diiradiasi pada dosis 10 kGy. Sampel tersebut kemudian diekstraksi dengan Na_2CyDTA, dan difraksionasi lalu dipisahkan untuk mendapatkan substansi pektin, hemiselulosa dan selulosa. Fraksi tersebut selanjutnya dianalisa untuk menentukan kadar gula netral, asam uronat dan kandungan selulosa, elusi polisakarida bermuatan, dan distribusi berat molekul. Hasil yang diperoleh menunjukkan bahwa iradiasi dinding sel polisakarida pada dosis 10 kGy dapat memecahkan makro molekulnya menjadi komponen yang lebih sederhana. Beberapa ikatan glikosida mengalami pemecahan akibat radiasi yaitu terlihat dari peningkatan jumlah komponen gula yang terlarut dan menurunnya berat molekul. Pola dan komposisi produk radiolisis serta derajat kepekaan jenis polisakarida tertentu yang dianalisa sangat bergantung pada kondisi lingkungan tertentu seperti bentuk dari dinding sel, orientasi fisik dari molekul polisakarida, dan lain-lain.

Kata kunci : iradiasi, daging buah mangga, isolasi dinding sel.

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EFFECT OF GAMMA IRRADIATION ON ISOLATED CELL WALL OF MANGO (*Mangifera indica* L.) var. Tommy Atkins PULP

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ABSTRACT

EFFECT OF GAMMA IRRADIATION ON ISOLATED CELL WALL OF MANGO (*Mangifera indica* L.) var. Tommy Atkins PULP. 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 of fresh fruit at pasteurization dose might create an adverse effect on the fruit texture by loosing 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 partly be elucidated by studying degradation products resulting from irradiation of the isolated cell wall of the fruit pulp. Cell wall polysaccharides were isolated from mango pulp by ethanol insolubilization prior to radiation dose at 10 kGy. A sequential extraction using Na_2CyDTA was applied to the treated samples followed by fractionation and isolation steps to obtain pectin, hemicellulose, and cellulose substances respectively. These fractions were analyzed in order to determine their neutral sugars, uronic acid and cellulose contents, elution behaviour of the charge polysaccharides, and molecular weight distribution. The results revealed that ionizing radiation at a dose of 10 kGy subjected to isolated cell wall polysaccharides could degrade macromolecules into smaller components. Some of the glycosidic linkages were split by irradiation as resulted by increasing some solubilized sugar components and the reduction of molecular weight. Pattern and composition of the radiolytic products and degree of sensitivity of these types of polysaccharides likely depending on the particular environments such as type of cell wall, physical orientation of the polysaccharides molecules, and of other artifacts.

Key words : irradiation,isolated cell wall, mango pulp.

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INTRODUCTION

Fruit production in Indonesia increased from 4.533 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 (1). Mango is one of the exotic tropical fruits which could also be promoted as non-oil export commodities to increase the country devisa. Export value of mango from Indonesia to various importing countries in the world showed an increase from US $ 579,000 in 1990 to US $ 857,000 in 1992 (2).

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.

Gamma irradiation as an alternative preservation method can be used to extend the storage life of some fruits and vegetables for different purposes (3, 4, 5, and 6). Adverse physico-chemical changes occuring in irradiated fresh fruits and vegetables using medium radiation doses are surface and flesh darkening as e result of enzymatic browning, and immediate softening due to the degradation of long chain polysaccharides (7, 8, 9, and 10). Purwanto and Maha (11), and Lazan and Ali (12) reported that an extensive softening in mango appears to be related to more extensive modification of the wall pectins. 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 (7,8,13, 14, 15, and 16). Fresh fruits such as mango is normally exposed to irradiation as constituents coexisting major and minor components of complex fruit systems. This study was aimed to obtain some information on degradation

products of isolated cell wall polysaccharides in mango fruit induced by irradiation as an approach to elucidate their degradation mechanism.

MATERIALS AND METHODS

1. Preparation of cell wall material

Mango (*Mangifera indica* L.) var. Tommy Atkins at commercial maturity stage (1.3 kg pulp) was extracted using ethanol. The alcohol insoluble solids (AIS) was prepared by precipitation of the wall in alcohol, ethanol, according to Heuthink (17) followed by washing the tissue with 70% ethanol until sugar free as indicated in the AIS samples as described by Dubois et al. (18).

2. Experimental design and sampling methods

The statistic method used was complete randomized design in triplicates according to Steel and Torrie (19), and diagonal sampling method as described by Lees (20). AIS was partly suspended in water (1:1), stirred, and kept over night at room temperature prior to radiation exposure in order to study radiation induced-degradation on solute polysaccharides in vitro.

3. Irradiation treatment

AIS as non-suspended, powder form, and as suspension, were irradiated at ambient temperature (20±25°C) using Co-60 at the Institute for Atomic Sciences in Agriculture (ITAL) Wageningen, The Netherlands. The dose rate applied was 6. 3 kGy/h.

All samples were then extracted with Na_2CyDTA solution in order to obtain depectinated cell wall materials, viz. Na_2CyDTA Insoluble Residue (CyDTAIR) and Na_2CyDTA Soluble pectin ($Na_2CyDTASP$) respectively according to the method of Renard et al. (21). The extracted samples were adjusted to pH 4.8-5.0 to prevent β-eliminative degradation of pectic material prior to the next fractionation. CyDTAIR was fractionated in mixtures of 50mM NaOH solution, and 4M NaOH containing 0.26 M $NaBH_4$ to obtain Alkali Soluble Pectin (ALKSP). This fraction mostly contained of hemicellulose and cellulose (22 and 23). The extraction scheme is illustrated in Figure 1.

4. Analytical methods

a. Cellulose content

Cellulose content of AIS and CyDTAIR were determined respectively based on anthron reaction according to Up de Graff method (24).

b. Uronic acid content

Uronic acid content in the samples was determined by a colorimetric m-Hydroxyl diphenyl assay and anhydrogalacturonic acid was applied as a standard solution (25).

c. Neutral sugar analysis

Fractionated CyDTAIR was further treated with aqueous 72% w/w H_2SO_4 for 1 h at 30°C followed by hydrolysis with 1 M H_2SO_4 for 3 h at 100°C. This method is to convert the individul sugar residues into alditol acetates. Inositol ws used as internal standard as described by Englyst and Cummings (26).

d. Gas Liquid Chromatography (GLC)

The alditol acetates was determined using chromatography method (27) in a glass column (3mm 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 at 270°C. Nitrogen was used as carrier gas.

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

The elution behaviour of the charge polysaccharides on an anion-exchange column was conducted according to the method of Schols et al.(28). 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 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 base line signal was corrected by substracting 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 x7.5 mm) in series (60XL,40XL, and 30XL; Biorad Labs) in combination with a TSK XL guard column (40 x 6 mm) and eluted at 30°C with 0.4M acetic acid/Na-acetate (pH 3.0) at 0.8ml/min. The eluent was monitored by a Shodex SE-61 Refractive Index Detector. Calibration of molecular weight was done using pectin as standard with molecular weight ranging from 10,000-100,000 Dalton. Samples (3-5 mg/ml) were solubilized in 0.4 M Na-acetate buffer at pH 3 and centrifuged prior to injection.

RESULTS AND DISCUSSION

Alcohol Insoluble Solids (AIS) of mango pulp as starting material was considered to be applied in this experiment as simple preparation on isolation of the fruit cell walls by removing undesired compounds using organic solvent. This isolation step extracts low

molecular weight sugars, amino acids, organic acids, and many inorganic salts and leaving behind an alcohol insoluble solids containing polymers. These polymers might include intracellular proteins, RNA and starch, but the contaminants can often be ignored (29). Study on the effect of irradiation on AIS as measured from cellulose content, sugar composition (g/100 g pulp) and anhydrouronic acid content resulted in marginal differences between control and irradiated samples with regard to cellulose and anhydrouronic acid content. No differences between control and irradiated samples were found in the amount of rhamnose, arabinose, xylose, mannose, galactose, and glucose contents.

Figure 2 illustrates the amount of pectin soluble in alkali (ALKSP), hemicellulose, and cellulose after extraction from irradiated AIS sample. It is presented in the same figure as in unirradiated AIS both in powder and suspended forms. These results were in agreement with other study as conducted by Rousse and Dennison (30). Water and oxalat soluble pectin showed an increase but protopectin prepared from NaOH fraction showed a decrease after irradiation. Similar results were obtained by Mc.Ardle and Nehemias (14) who studied the effect of gamma irradiation on pectic substances derived from alcohol insoluble solids of apple and carrot. These supporting results might confirm that in all cases a break down of protopectin into pectate and soluble pectin has occured. A decrease of total pectic substances i.e., pectin, pectate and protopectin indicates a breakdown of pectin and pectate into simpler compounds as non pectic materials. The most extensive changes has occured in the protopectin constituent which was almost completely destroyed by the highest radiation dose applied in their experiment. The ALKSP content in the supernatant and mild alkali extraction of suspended AIS showed a decrease after

irradiation but on the other hand hemicellulose content in the same samples showed an increase after irradiation. Irradiation of the suspended AIS resulting a decrease in cellulose content. Similar result was reported by Roe and Bruemmer (31) in the study on enzymic activity of mango pulp. The result revealed that loss of firmness in the fruit pulp was induced by the increasing activity of endo polygalacturonase and cellulase as indicated by a reduction in water and alkali soluble pectin content in the pulp. The same treatment showed a remarkable increase in ammonium oxalate soluble pectin content in the pulp. The effect of gamma irradiation on C_7DTAIR fraction derived from non-suspended AIS is illustrated in Figure 3. It is shown that there was not any significant changes in the sugar composition after irradiation except for Anhydrouronic acid content (AUA).

Results of sugar composition on freeze dried of dialyzed-supernatant both non suspended and suspended AIS before and after irradiation at 10 kGy then extraction with Na_2CyDTA is illustrated in Figure 4. It is shown that there was not any changes on sugar content in the Na_2CyDTASP sample. A remarkable result was obtained in unirradiated sample of suspended AIS. The anhydrouronic acid content showed an inrease about 50% after irradiation. The sugar content of the extractable pectin were small although contrary to the result of CyDTAIR fraction (Figure 3) which mostly contained arabinose and glucose. A comparable result was by Voragen et al. (22). The highest content of saccharides in ethanol soluble residue of mango pulp were galacturonic acid and glucose. Arabinose was high in pectin fraction. The most important constituent in hemicellulose of mango pulp are xylans, mannans or glucomannans and xyloglucans. Extracted guinean mango pulp using HCl at pH 5 and at a temperature of 85°C resulted in obtaining of galacturonic acid 40-70%, arabinose 2-4%, rhamnose 1-2%, xylose 1-7%, mannose 1-3%,

galactose 14-22% and glucose 8-22% (32). Contrary result was reported by D'Amour et al.(33). Irradiated fresh strawberries at the dose of 4 kGy showed a decrease in galacturonic acid content as extracted from water insoluble pectin and hemicellulose fraction respectively meanwhile the neutral sugars originated from the pectin side chains were not influenced by irradiation. Studies on the effect of enzyme activity on the ripening step of unripe into ripening stage in mango was demonstrated by Simpson et al.(34). It showed that in the unripe stage, pectin embedded in a complex matrix with cellulose as protopectin. In the frseh unripe fruit this pectin is protected by cellulose against pectolytic degrading enzymes or other fruit constituents.Pectin content seems to increase from immature green stage to its peak value in the mature green stage, and the 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 shows an increase in soluble pectin. Such degradation process is comparable to radiation treatment in certain condition.Hemicellulose fraction derived from irradiated and non irradiated suspended AIS in water is illustrated in Figure 5. The result revealed that total xylose and glucose contents in hemicellulose fraction showed 50% increase after irradiation. The increasing sugar content is probably due to the fact that xyloglucans are more easily extracted after irradiation both in powder and suspended AIS. The contents of the other sugars and anhydrouronic acid in all fractions are not significantly affected by irradiation. Results on the elution behaviour of charge polysaccharides in irradiated soluble pectins as measured by HPIEC revealed that there is no shifting of the elution profiles into earlier retention time. This results might illustrate that there is no changes on the distribution of methoxyl

groups gives an indication that there was not elimination reaction on the β-position (Figure 6).

Figure 7 illustrates the effect of gamma irradiation on molecular weight distribution of Na_2CyDTASP fraction derived from non-suspended AIS as measured by HPSEC. The chromatogram clearly demonstrates a reduction in the molecular weight of the polysaccharides. It can be explained that elution of the molecules shifted into longer retention time after irradiation. It is evident that some degradation within molecular chains has occured in 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. It seems that shifting the main peak results in increasing the concentration of lower molecular weight after irradiation as it is shown by an increase of shoulder peak. This situation may be already the case in the unirradiated sample where treatment may result in a shift to lower molecular weight for both populations. Variability in the range of molecular weight of different HPGPC columns in series might contribute to the separation. The TSK 40XL column does not separate oligomers but only separate molecules between 10-20,000 Dalton and 100,000 Dalton for pectin. TSK 30XL column separates mono and oligomers, and also capable to separate molecules up to 60,000 Dalton but the resolution is poor. The same result was obtained from the non-suspended AIS. Neutral sugars, AUA and cellulose contents of AIS showed marginal changes after irradiation.

It is well understood that the presence of some radical scavangers as proton donor such as vitamin and protein in the fresh fruit might create a complex matrix. This situation initiate some difficulties in order to elucidate a degradation mechanism as well as immediate softening in fresh fruit following radiation.

Effect of gamma irradiation on alcohol insoluble solids has been reported. Remarkable decrease in pectin, and cellulose contents were demonstrated both in non suspended and suspended AIS. These results were in agreement with Sonntag (35). Some neutral sugars such as xyloglucans show an increase in the fractions of irradiated suspended AIS such as CyDTAIR, Na_2CyDTASP, and hemicellulose. Xyloglucans are hemicellulose fraction which absorb into the cellulose fibrils and as cementing agent between those fibrils. Gamma irradiation induces degradation on xyloglucan and resulting losses of cementing function which directly reflects to the fruit texture. The mechanism proposed for irradiation of methoxyl pectin in solid state as direct effect reaction predominant is illustrated in Figure 8.

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 elucidate a degradation mechanism in irradiate fruit induces immediate softening. It is probably due to the complexicity of isolated cell wall polysaccharides of AIS as starting materials, which contains some unreleased contaminants such as intra cellular proteins, RNA and starches. These contaminants might interact during degradation process after irradiation of the AIS samples.

CONCLUSION

It is obvious from the finding results that gamma irradiation induces degradation on isolated cell wall components of mango pulp, i.e., pectin, and hemicellulose both in solid and solute states. The conversion of insoluble wall-bound protopectin of high molecular weight into water soluble pectin was remarkable by reducing a number of molecular

weight. Immediate softening of fruit after irradiation which is considered as an adverse effect, can be correlated with the degradation of the wall components in solute state.

Effect of gamma irradiation at the dose of 10 kGy on isolated cell wall polysaccharides of mango by alcohol precipitation as starting material showed marginal changes on neutral sugars, except in xyloglucans, anhydrouronic acid, and cellulose contents. Sugar composition of insoluble residue after extraction with Na_2C_YDTA did not change upon irradiation except for anhydrouronic acid which was virtually absent after irradiation.

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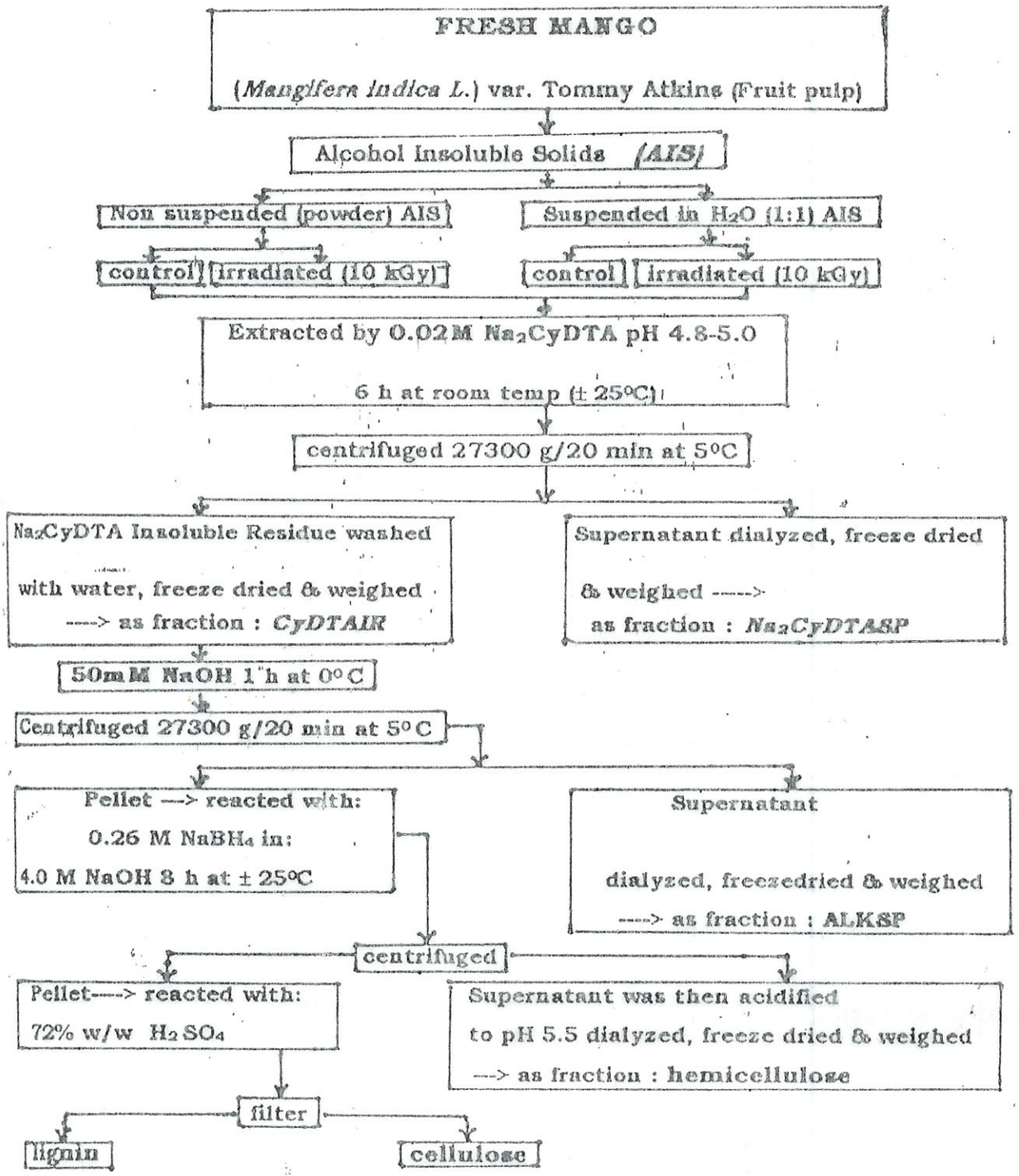

Figure 1. Extraction scheme of cell wall polysaccharides in mango *(Mangifera indica L.)* var. Tommy Atkins

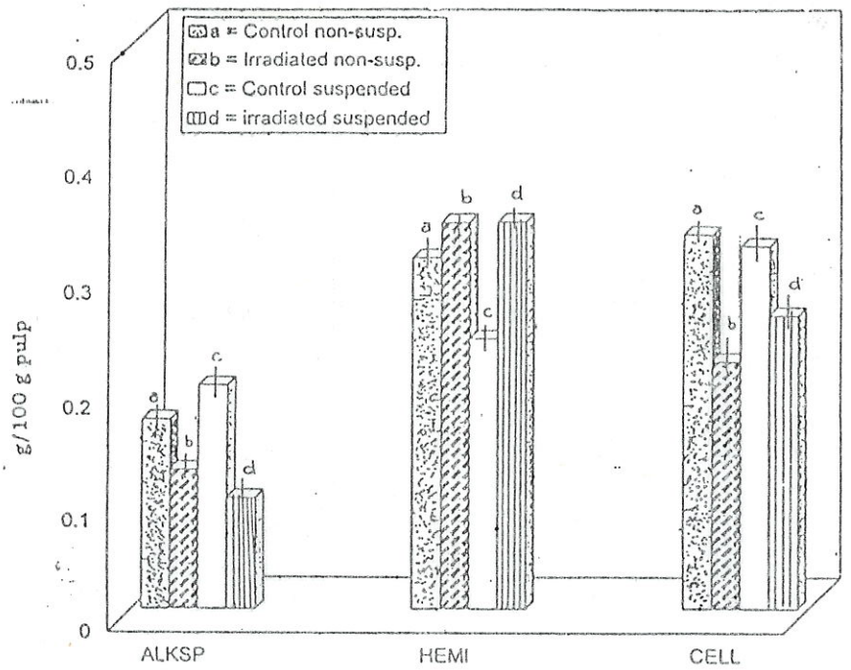

Figure 2. Effect of irradiation at 10 kGy on non-suspended and suspended AIS as measured for ALKSP, hemicellulose and cellulose from Na_2CyDTA extracted samples

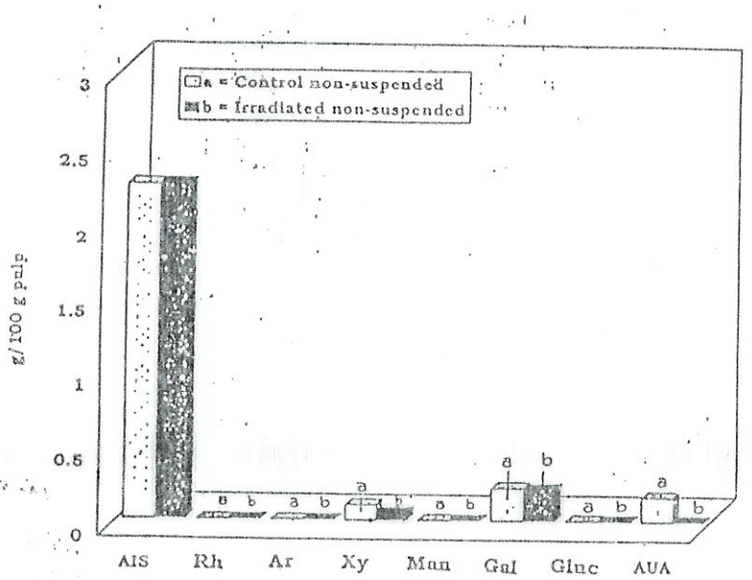

Figure 3. Effect of irradiation at 10 kGy on non-suspended AIS as measured for sugar composition and anhydro-uronic acid content in CyDTAIR fraction

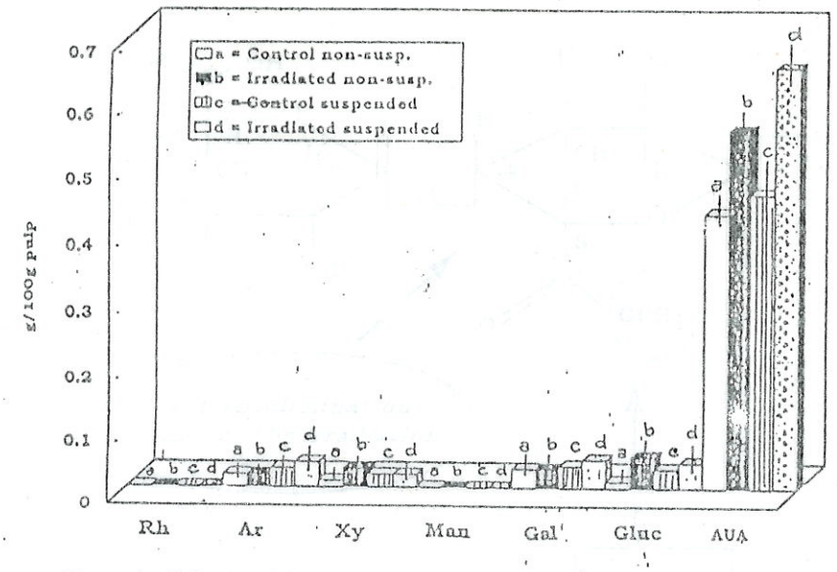

Figure 4. Effect of irradiation at 10 kGy on non-suspended and suspended AIS as measured for sugar composition and anhydrouronic acid content on $Na_2CyDTASP$

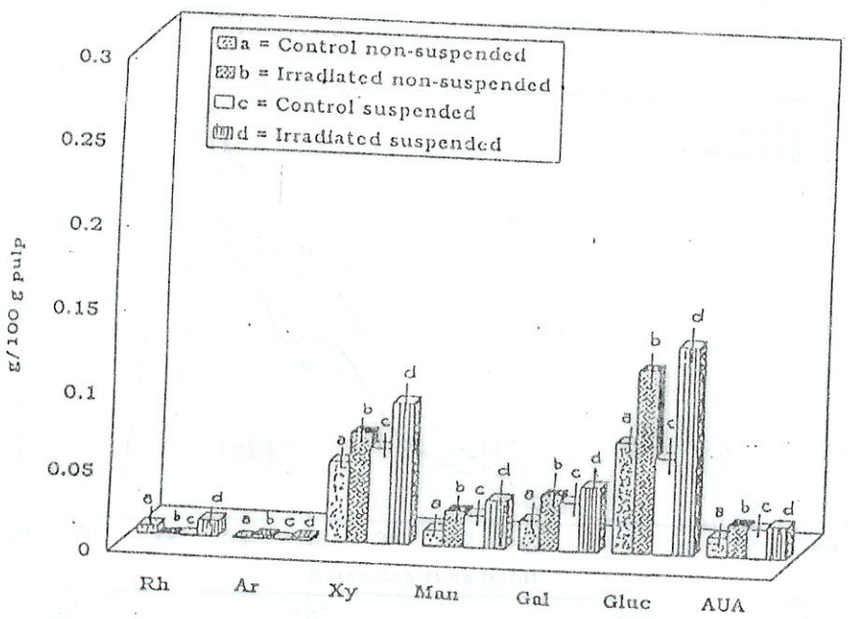

Figure 5. Effect of irradiation at 10 kGy on non-suspendedand suspended AIS as measured for sugar composition and anhydrouronic acid content on hemicellulose fraction

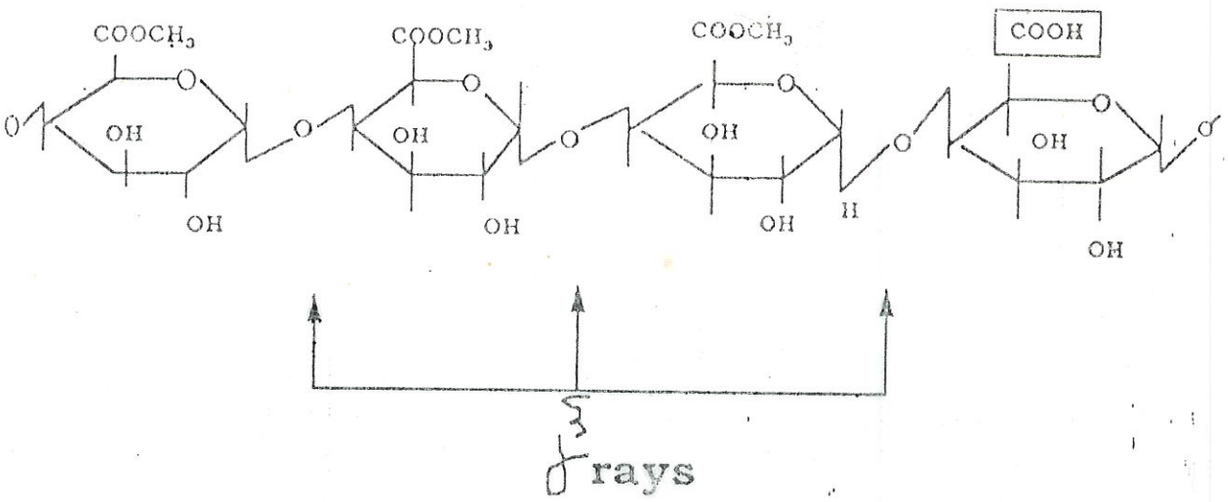

Figure 8. Proposed mechanism for irradiation of high methoxyl pectin at direct effect reaction predominant