Radiation Effects on Pectinase Solutions[1]

Pectinase is the enzyme which catalyzes the hydrolysis of the 1–4-glycosidic linkages in the polygalacturonic acid with the resulting formation of (mono)-galacturonic acid and of polygalacturonic acid having smaller molecular sizes. Since this enzyme plays a very important role in the pectic metabolism and during the ripening of fruits[2–4], our research was undertaken to investigate the sensitivity of the enzyme to ionizing radiations. The work consisted of the irradiation of aqueous solutions of pectinase with several concentrations of pure enzyme subjected to various doses of irradiation.

A Stabilipan X-ray machine operating at 250 kV (50 R/min $\lambda = 0.16$ Å) was used to irradiate the solutions. The doses given were 0.5 and 1 Mrad for all concentrations while, for $1.5 \times 10^{-3} M$ aqueous solutions, the following doses were given: 1 Krad, 10 Krad, 50 Krad, 200 Krad, 500 Krad and 1 Mrad. The irradiation was made in 20 cm diameter Petri dishes.

The commercial pectinase (Schuchardt P.M. 176n) was purified by chromatographic separation on a 1.5 cm diameter column filled with 35 cm of a gel Sephadex G-50 fine activated in $0.1 M$ phosphate buffer pH 6. Fractions containing 10 cm of eluate were collected and the enzymatic activity was determined for each fraction. Such activity increased in the first fractions and decreased rapidly after reaching the maximum. The fraction of the purified enzyme having the highest activity was irradiated. As the pectinase splits the polymers of the galacturonic acid into others less polymerized, increasing, as a result, the free reducing groups, the determination of activity is based either on the formation of these reducing groups[5,6] or on the decrease of viscosity produced by the polymerizing effect. In the last case, the method of determination is quick and of easy application but not too specific. The Folin's method, once modified, was used to measure the reducing groups. Such a method is sensitive as well as specific at low activities and it utilizes the power of galacturonic acid and of its polymerized shapes to reduce alkaline copper solutions. Consequently, the reduced copper is put into evidence by addition of phosphomolibdic reactive which forms a blue compound with the reduced copper. The enzyme activity is determined at the temperature of 30 °C in a mixture containing 5[3] cm of purified enzyme and 50 cm[3] of polygalacturonic acid 0.5% pH = 4.5. 1 cm[3] of the mixture is diluted 100 times with H_2O and 1 cm[3] of Folin (reagent rameic Medi-Chem-Inc) reactive is added to 1 cm[3] of the diluted mixture in a test-tube. The test-tube is placed for 7 min in a boiling water bath. After cooling, 1 cm[3] of the Folin (reagent phosphomolibdic Med-Chem-Inc) reactive is added and placed for 3 min in a boiling water bath. The test-tube is cooled again and 10 cm[3] of H_2O are added.

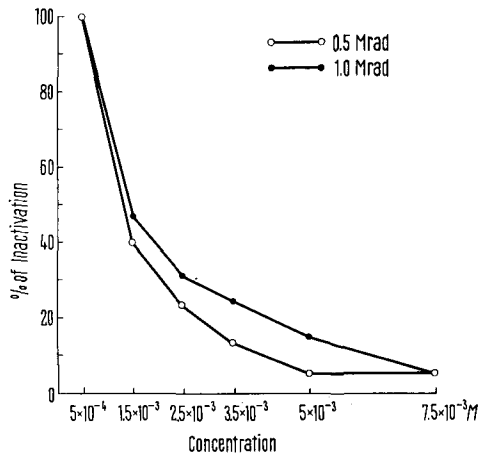

Fig. 1. Inactivation of enzyme activity at doses of 0.5 and 1 mR against enzymatic solution.

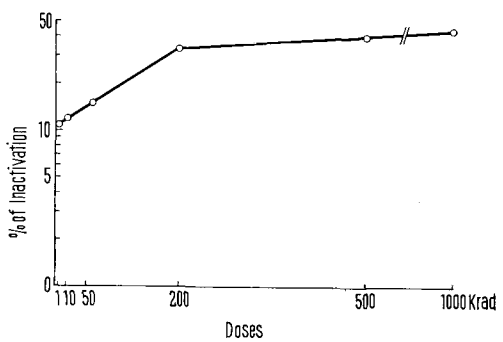

Fig. 2. Enzymatic inactivation at $1.5 \times 10^{-3} M$ concentration.

The optical density is determined at $\lambda = 620$ nm against a blank. In order to calculate the amount of the reducing groups from the optical density values, a standard curve is prepared by increasing amounts of galacturonic acid up to a maximum of concentration of 0.2g/100 ml, the Lambert-Beer's law being followed.

Figure 1 shows the percentages of inactivation of enzyme activity at the doses of 0.5 and 1 Mrad against the concentration of the enzymatic solution. The inactivation of the enzyme does not change according to the concentration. For a concentration of $5 \times 10^{-4} M$ the inactivation is total, while for slightly higher concentrations up to $1.5 \times 10^{-3} M$ there is a considerable drop. Consequently, the curve drops showing a different slope. In the first part of the curve, corresponding to the lowest concentrations, the indirect effect is prevalent; in the second part, corresponding to the highest concentrations, the effect decreases and it is not counterbalanced by the increase of the direct effect which is less than 7%. The 2 doses of 0.5 and 1 Mrad inactivate the enzyme in different measure. The doses of 0.5 Mrad at $5 \times 10^{-3} M$ concentration gives a very low effect which keeps constant also at higher concentrations. This confirms that the direct effect on the enzyme is very low. When a dose of 1 Mrad is given to the enzyme, the indirect effect on the enzymatic activity extends to higher concentrations. The half-inactivated

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pectinase concentration at the doses so far considered was examined to know the effects of doses lower than 0.5 Mrad on enzymatic activity. In Figure 2 the percentages of enzymatic inactivation at $1.5 \times 10^{-3} M$ concentration as function of 1, 10, 50, 200, 500 and 1,000 Krad doses are reported.

At the dose of 1 Krad, the enzyme is still sensitive and such sensitivity increases exponentially until 200 Krad, tending, afterwards, to asymptote.

These results, showing the sensitivity of the enzyme also at low doses, seem to be promising for the application of ionizing radiations to fruit preservation, and the work can be considered as a preliminary research dealing with the inactivation of in vivo pectinase[7].

Riassunto. È stata misurata la radiosensibilità dell'enzima Pectinasi a più concentrazioni e a varie dosi di irraggiamento. Si descrive inoltre un nuovo metodo per determinarne l'attività.

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Concerning Collagen and Elastin in Aortas of Nine Different Species

A large number of studies related to the elastin and collagen content of human or animal aortic tissue have been using histological techniques, but only relatively few quantitative chemical studies have been accomplished[1–5]. It could be mentioned that HARKNESS et al.[5] studied the relative distribution of elastin and collagen in dog aorta from the arch to the lower abdominal segment. A similar study using aortas of goat, sheep, pig and human was conducted by GRANT[6,7].

In our study, elastin hydroxyproline (elastin HP) content and collagen hydroxyproline (collagen HP) content in aortas of 9 different healthy species were determined. In 5 of these species aortas were separated into abdominal and thoracic segments prior to the hydroxyproline determination.

The elastin HP and collagen HP were also determined in aortas of a New Zealand albino rabbit subjected to an atherogenic diet for 0, 3 and 7 weeks respectively. It was hoped that this experimental study, on one hand, would shed some light on the aortic elastin and collagen content in 8 different species of mammals not examined previously but often employed as experimental animals in cardiovascular research; on the other hand it was hoped that this study could relate changes in elastin HP and collagen HP in rabbit aorta to the atherosclerotic involvement of aorta during development of dietary atherogenesis.

Materials and methods. Nine different animal species (of mixed sexes) were employed in this study. Aortas of mice, rats, golden hamsters, guinea-pigs, cats, dogs and rhesus monkeys were obtained from Pel Freez Biological Inc., Rogers, Arkansas. Bovine aortas were obtained through Dr. I. LIKAR from a commercial slaughter-house, Worcester (Mass.). Rabbits (New Zealand, albino) were obtained from Glocester Rabbitry, Glocester (Rhode Island). Three groups of rabbits (10 rabbits each) were fed an atherogenic diet (Purina rabbit chow fortified with 1% cholesterol) for 0, 3 and 7 weeks. At the end of these periods, rabbits were sacrificed, aortas were separated into thoracic and abdominal segments and the areas of the atherosclerotic lesions were determined planimetrically.

Further experimental procedures were identical in all investigated species. Aortas were stripped of their adventitial layers, separated into abdominal and thoracic sections in the rabbit, cat, dog, monkey and cow (and into intima and media in case of bovine aorta), dehydrated in several changes of acetone and dried in vacuum oven (temperature < 50 °C). Portions of dry tissue (5–15 mg) were accurately weighed and homogenized in 15 ml of distilled water; collagen and elastin were separated and hydroxyproline content determined in both fractions. The experimental procedure was essentially the same as recently described by GRANT[7]. All results are expressed as milligrams of hydroxyproline in collagen or elastin fractions, respectively, per gram of dry tissue. Ten mouse aortas were combined per sample.

Table I. Determination of collagen hydroxyproline and elastin hydroxyproline in aortas of 9 species of animals

Species	No. of animals	Hydroxyproline (mg/g dry tissue) Collagen fraction	Elastin fraction
Mouse	50	17.9 ± 0.7[a]	3.1 ± 0.3
Rat	20	26.5 ± 1.2	4.4 ± 0.6
Hamster	10	22.4 ± 1.9	7.4 ± 0.5
Guinea-pig	20	16.2 ± 1.1	3.3 ± 0.4
Rabbit	10		
Thoracic part		18.0 ± 1.9	5.5 ± 0.6
Abdominal part		25.8 ± 7.2	4.9 ± 0.7
Cat	10		
Thoracic part		23.5 ± 0.9	4.3 ± 0.2
Abdominal part		31.9 ± 1.3	4.1 ± 0.4
Dog	10		
Thoracic part		19.5 ± 2.0	4.4 ± 1.0
Abdominal part		34.9 ± 2.3	3.8 ± 0.5
Cow	10		
Thoracic intima		20.6 ± 2.0	6.3 ± 0.8
Thoracic media		22.9 ± 1.5	6.0 ± 0.5
Abdominal intima		27.7 ± 3.8	6.5 ± 0.7
Abdominal media		38.6 ± 4.4	5.4 ± 0.5
Monkey	10		
Thoracic part		25.1 ± 0.8	4.7 ± 0.3
Abdominal part		30.6 ± 1.1	4.3 ± 0.2

[a] Mean ± S.D.