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Biochemical changes in some tropical fruits during ripening

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Changes during ripening in alcohol-insoluble solids (AIS) and dietary fibres of mango, guava, date, and strawberry as tropical fruit are described. The activities of various degradative enzymes in the fruits were also investigated. The results showed that the AIS and texture declined rapidly during ripening. The dietary fibres decreased as the fruits lost their firmness and became soft. Polygalacturonase and cellulase activities of the fruit tissues increased markedly during ripening in mango, guava, and strawberry fruits. Both polygalacturonase and cellulase were absent or present at only a low level in the green date but displayed large increases in activity during ripening. The changes in polygalacturonase and cellulase activity were reflected in a reduction in fruit firmness and a decrease in anhydrogalacturonic acid (AGA) and cellulose content during ripening. Pectinesterase (PE) activity decrease in mango, guava, and strawberry fruits during ripening, but its activity was increased in date during ripening, although the degree of esterification (DE) of pectin decreased.

INTRODUCTION

Texture is an important characteristic of food, and control or modification of texture is a major objective in modern food technology. The handling and processing of fruit and vegetables involves special problems, since the consumer has well-formed opinions and expectations regarding the proper texture in these commodities. Fruits soften during maturation and storage. These changes relate to changes in their cell-wall components. However, these changes are accompanied by solubilization of pectin (Huber, 1983).

Sobotka and Stelzig (1974) reported that cellulose occurs in orderly arrangements throughout the primary and secondary walls of plant cells, and it might be expected that cellulose should play a key role in texture changes.

The galacturonan chains in pectin are also cleaved by poly-α-1,4-galacturonic glycanohydrolase, EC 3.2.1.15, which is usually referred to as polygalacturonase (PG). This enzyme is produced by micro-organisms and by higher plants. Polygalacturonase has been found in mangoes (Roe & Brummer, 1981), in guavas (Augustin *et al.*, 1985), in dates (Mustafa *et al.*, 1986), and in strawberries (Huber, 1984). Polygalacturonases are specific for de-esterified galacturonans, i.e. polygalacturonic acid. The rate and extent of hydrolysis by polygalacturonase are functions of the degree of de-esterification of pectin (Jensen & MacDonnel, 1945).

It has been suggested that at least two adjacent free carboxyl groups are necessary for polygalacturonase action to occur (Jensen & MacDonnel, 1945). This requirement for de-esterification suggests that pectin hydrolysis in plants may be controlled by the enzyme pectinesterase. However, evidence has not been provided that pectinesterase is the controlling factor in fruit-softening (Hosbon, 1963).

The pectinesterase has been investigated in mango (Ashraf *et al.*, 1981; Roe & Brummer, 1981), in guava (Mowlah & Itoo, 1983; Augustin *et al.*, 1985), in date (Mustafa *et al.*, 1986), and in strawberry (Gizis, 1964).

It has been suggested that cellulase, in addition to pectic enzymes, may contribute to the softening of fruit during ripening. Data from numerous reports clearly show that plant cell walls can be digested by cellulase. These changes occurred in mango (Roe & Prummer, 1981), in guava (Mowlah & Itoo, 1983), and in date (Hasegawa & Smolensky, 1971).

The aim of this study was to investigate the relationship between loss of firmness and cell-wall components and to investigate the enzyme activities that are involved in the ripening process.

MATERIALS AND METHODS

Materials

Fruit was obtained from Ismailia farms (Egypt) as follows: two varieties of mangoes were used, namely

Zebda and Baladi. Mangoes were picked at the green-mature, half-ripe and ripe stages from trees. Guava were also picked at green-mature, half-ripe, and ripe stages. The Baladia variety of guava was used. Dates (Hayani variety) were picked from trees at green-mature, red, and black (Rutab) stages. The Tioga variety of strawberry was used, and the fruits were picked at the white, pink, and red stages of ripening. After textural studies on representative fruit from each stage, half of the remainder of the fruit was frozen in liquid nitrogen and stored at −20°C. Frozen fruit was employed for alcohol-insoluble-solids (AIS) and enzyme analysis, and freeze-dried fruit was used for pectin and other dietary fibre analysis. For the determination of dry weight, fruit was dried to constant weight.

Methods

Shear-resistance assessment
Tissue firmness was estimated from shear-resistance measurements obtained with an Allo Kramer press, Model SP-121MP (Allo Precision Metals Engineering Inc., USA, Rockville, MD). Slices of fruit tissues (8 mm × 85 mm^2) were taken from each test fruit and placed in the 65-cm^2 cell box. The plunger stroke was adjusted to a control speed of 3·8 cm/min. Tissue resistance in lbf was measured and recorded with an electronic transducer ring with a range of 0–10 lbf. All values were expressed as lbf per 32-mm-thick sample (four slides).

Preparation of alcohol-insoluble solids
Alcohol-insoluble solids (AIS) were prepared by the method of El-Zoghbi (1989). Frozen fruit (40 g) were blended and added to 200 ml of boiling ethanol and boiled for 30 min. After it had been cooled to room temperature, the mixture was filtered through a Büchner funnel. This was repeated several times until no free sugars were found in the extract. The residue was washed with ethanol and then with acetone and dried to constant weight in a vacuum oven at 50°C.

Extraction of pectin fractions
Pectin was extracted from samples by the method of Voragen *et al.* (1983).

Anhydrogalacturonic acid determination
The anhydrogalacturonic acid (AGA) content of pectin was determined according to the method of Ahmed and Labavitch (1977).

Determination of degree of esterification (DE)
The degree of esterification of pectin was determined titrimetrically according to El-Zoghbi (1989).

Determination of fibre
Neutral-detergent fibre (NDF), acid-detergent fibre (ADF), acid-detergent lignin (ADL), and ash were determined in sequence from the same samples (2 g) of ground freeze-dried fruit as described by Van Soest (1963) and Van Soest and Wine (1967) by using the Tecator Fibertec (Tecator Ltd, Bristol, UK). Values recorded are the means of duplicate analyses. Approximate fibre fractions were calculated as: hemi-cellulose = NDF − ADF, cellulose = ADF − ADL, lignin=ADL − ash.

Cellulose content
Cellulose content was determined according to the method described by Updegraff (1969).

Enzymes extraction
Fruit tissue (50 g) was homogenized with 50 ml of a cold solution of 12% poly(ethylene glycol) (Carbowax 6000) and 0·2% sodium bisulfite (pH 5) in water for 1 min in an Ultratex homogenizer. The suspension was centrifuged at 7000 *g* for 20 min under cooling for recovery of the residue. The residue was washed twice by homogenization in 100 ml of cold distilled water followed by centrifugation. The residue was suspended in 50 ml of 0·5 M NaCl. This suspension constitutes the enzyme preparation for assay of polygalacturonase, pectinesterase, and cellulase.

Polygalacturonase (E.C.3.2.1.15) assay
Polygalacturonase activity was determined by a modification of a procedure of Pressey *et al.* (1971). The previous enzyme suspension (2 ml) 0·8 ml of 0·2 M sodium acetate buffer (pH 4), 2 ml of 1% sodium polygalacturonate (pH 4), and 100 μml of 0·2% sodium azide were placed in a 50 ml Erlenmeyer flask. An identical sample heated in boiling water for 3 min served as a blank. Sodium azide was added to inhibit growth of micro-organisms. The samples were incubated at 30°C in a shaking incubator. After 19 h, the samples were inactivated in boiling water for 3 min, cooled, and centrifuged. The supernatants were analyzed for reducing groups by the method of Honda *et al.* (1982). A unit of PG is the amount of enzyme that catalyzes the release of 1 μmol of reducing groups in 19 h at 30°C.

Pectinesterase (E.C.3.1.1.11) assay
Enzyme suspension (10 ml) was assayed for pectinesterase by the method of Vas *et al.* (1967). One unit of PE is the amount of enzyme that liberates one μmol of carboxyl groups per minute.

Cellulase (E.C.3.2.1.4) assay
Enzyme suspension (1 ml) was added to 3 ml of 0·5% carboxymethylcellulose and 1 ml of 0·1 M sodium citrate–phosphate buffer (pH 6·0). An identical sample heated in boiling water for 3 min served as control. Sodium azide (100 μml, 0·2%) was added to the samples. The samples were incubated at 30°C for 19 h, and then inactivated in boiling water for 3 min. After the samples had been cooled and centrifuged, 1 ml of

supernatant was analyzed for reducing sugars by the method of Honda *et al.* (1982). One unit of cellulase activity was defined as the amount of enzyme that catalyzes the formation of 1 μmol reducing groups in 19 h at 30°C.

RESULTS AND DISCUSSION

Softening of fruit flesh as indicated by the decrease in resistance to shearing force was accompanied by a rapid decrease in the amount of alcohol-insoluble solids (AIS) recovered from all fruits investigated (Table 1). From a highest value of 7·8% in green date, the AIS decreased to 4·0% of fresh weight in the black stage of ripening. In all fruits investigated, AIS also decreased as the fruit ripened. Most of this decrease may be due to the conversion of starch to soluble sugars and also to conversion of the fibre to alcohol-soluble solids. Subramanam *et al.* (1972) reported that, as mangoes ripened, the AIS declined rapidly, with a concomitant increase in sugar contents and total soluble solids. Many investigators also showed that, during fruit-ripening, the AIS content declined in mango and guava (El-Zoghbi, 1989), in date (Mustafa *et al.*, 1986), and in strawberry (Huber, 1984).

The fibre components at different stages of ripening in mango, guava, date, and strawberry fruit are presented in Table 2. The amount of fibre, as a percentage of fresh weight, decreased with maturation in all fruits investigated. The decline was in all dietary-fibre fractions. The percentages of the decrease of total fibre were about 84·2, 74·0, 74·3, 71·3, and 72·6% from the original amounts of guava, date, mango var. Baladi, mango var. zebda, and strawberry, respectively. This decrease can be due to hydrolysis in the cell wall by the native enzymes, e.g. pectinase, hemicellulases, and cellulases. On the other hand, the date fruit has the highest percentage of total fibres followed by guava, mango, and strawberry fruit, respectively.

The degree of esterification (DE) of pectin and pectinesterase activity of the fruit investigated is given in Table 3. Pectinesterase activities of the fruit tested were found to decrease during ripening except for dates. Roe and Bruemmer (1981) reported that the pectinesterase activity of Keitt mangoes declined during ripening. Many researchers also showed that pectinesterase activity decreased during ripening in oranges (Tahir *et al.*, 1975), bananas (Hultin & Levine, 1965), apples, tomatoes, and avocado (Bartley & Knee, 1982) and pears (Nagel & Patterson, 1967). PE activity of Sudanese (Mustafa *et al.*, 1986) and of Iraqi dates (Al-Jasim & Al-Delaimy, 1972) also increased during fruit ripening. Over all fruits investigated, the DE of pectin declined during ripening. The decrease in DE during ripening may be due to the action of PE.

Polygalacturonase (PG) activity and anhydrogalacturonic acid (AGA) content are presented in Table 4. The results showed that PG activity increased during ripening and was accompanied by a decline in AGA content and resistance to shearing force (see Table 1) of mango, guava, date, and strawberry fruit. These results

Table 1. The percentage of alcohol-insoluble solids (AIS) and resistance to shearing force of some tropical fruits

Fruit	Stage of ripening	AIS (%)	Resistance to shearing force (lbf)
Mango var. zebda	Mature	5·00	35·0
	Half-ripe	3·80	18·0
	Ripe	2·95	3·9
Mango var. Baladi	Mature	4·14	31·0
	Half-ripe	3·40	16·0
	Ripe	2·43	3·5
Guava	Mature	6·18	40·0
	Half-ripe	4·60	20·0
	Ripe	3·10	5·5
Date	Green	7·80	24·0
	Red	6·50	14·4
	Black	4·00	4·8
Strawberry	White	1·90	10·0
	Pink	1·40	4·0
	Red	1·00	2·4

Table 2. Dietary-fibre fractions of tropical fruits during ripening (expressed as g/100 g tissue)

Fruit	Stage of ripening	Pectin	Hemi-cellulose	Cellulose	Lignin	Total fibres*
Mango	Mature	1·50	1·35	2·46	0·96	6·27
var. zebda	Half-ripe	1·10	0·73	1·28	0·43	3·54
	Ripe	0·66	0·30	0·64	0·20	1·80
Mango	Mature	1·33	1·16	2·32	0·87	5·68
var. Baladi	Half-ripe	0·85	0·69	1·22	0·44	3·20
	Ripe	0·47	0·25	0·54	0·20	1·46
Guava	Mature	1·20	4·20	2·70	3·36	11·46
	Half-ripe	0·66	1·76	1·32	0·92	4·66
	Ripe	0·26	0·60	0·75	0·20	1·81
Date	Green	1·58	5·25	3·40	3·50	13·73
	Red	0·96	2·66	1·98	1·25	6·85
	Black	0·54	1·29	1·44	0·30	3·57
Strawberry	White	0·44	0·26	0·92	0·13	1·75
	Pink	0·27	0·17	0·63	0·07	1·14
	Red	0·14	0·10	0·22	0·02	0·48

*Total fibres = sum pectin, hemicellulose, cellulose, and lignin.

Table 3. Pectinesterase activity and DE% of pectin of some tropical fruits during ripening

Fruits	Stage of ripening	Enzyme activity (units/100 g tissue)	DE%
Mango var. zebda	Mature	23·2	85·0
	Half-ripe	19·3	80·0
	Ripe	15·0	72·0
Mango var. Baladi	Mature	42·4	72·0
	Half-ripe	33·9	68·0
	Ripe	28·0	64·0
Guava	Mature	31·9	87·0
	Half-ripe	28·0	79·0
	Ripe	26·3	73·0
Date	Green	20·0	63·0
	Red	30·7	60·0
	Black	60·8	48·0
Strawberry	White	12·0	35·0
	Pink	8·0	25·0
	Red	5·2	12·0

Table 4. Polygalacturonase activity and pectin content (as AGA%) of some tropical fruits during ripening

Fruits	Stage of ripening	Enzyme activity (units/ 100 g tissue)	AGA% (g/100 g tissue)
Mango var. zebda	Mature	106	0·90
	Half-ripe	205	0·80
	Ripe	347	0·71
Mango var. Baladi	Mature	38·9	0·73
	Half-ripe	42·0	0·65
	Ripe	50·7	0·58
Guava	Mature	60·4	0·69
	Half-ripe	68·7	0·58
	Ripe	73·1	0·53
Date	Green	0·0	1·50
	Red	30·0	1·30
	Black	100	1·00
Strawberry	White	18·3	0·20
	Pink	23·5	0·18
	Red	36·0	0·16

are similar to those for mango (Roe & Bruemmer, 1981), guava (Mowlah & Itoo, 1983; Simpson *et al.*, 1984) and date (Mustafa *et al.*, 1986). On the other hand, PG activity was absent in the green stage of date and then it rapidly increased at the red and black stages of ripening. The major increases in the activity exhibited by these enzymes immediately preceded the loss of firmness in the fruit.

Cellulase activity of fruit investigated increased during ripening (Table 5) and was accompanied by a decline in cellulose content. In addition to PG, cellulase is probably the degrading enzyme responsible for the softening of fruit during ripening. In peaches, ripening and tissue softening were related to a marked increase in cellulase and PG activity (Pressey *et al.*, 1971; Hinton & Pressey, 1974). Yusof *et al.* also (1988) reported that the cellulase content of guava decreased during ripening.

Table 5. Cellulase activity and cellulose content of some tropical fruits during ripening

Fruits	Stage of ripening	Enzyme activity (units/ 100 g tissue)	Cellulose content (g/100 g tissue)
Mango var. zebda	Mature	20·5	1·50
	Half-ripe	23·7	1·30
	Ripe	28·8	0·74
Mango var. Baladi	Mature	9·1	1·30
	Half-ripe	15·6	0·95
	Ripe	38·8	0·70
Guava	Mature	37·9	2·00
	Half-ripe	55·0	1·50
	Ripe	73·1	1·00
Date	Green	5·3	2·40
	Red	60·0	1·80
	Black	115·3	1·20
Strawberry	White	10·0	0·55
	Pink	20·3	0·40
	Red	33·1	0·20

REFERENCES

Ahmed, A. & Labavich, J. (1977). A simplified methods for accurate determination of cell wall uronide content. *J. Food Biochem.*, **1**, 361.

Al-Jasim, H. & Al-Delaimy, K. (1972). Pectinesterase activity of some Iraqi dates at different stages of maturity. *J. Sci. Food Agric.*, **23**, 915.

Ashraf, M., Kahn, N., Ahmed, M. & Elahi, M. (1981). Studies on the pectinesterase activity and some chemical constituents of some Pakistani mango varieties during ripening. *J. Agric. Food Chem.*, **29**, 526.

Augustin, M., Ghazal, H. & Hashim, H. (1985). Enzyme activities of guava fruits. *J. Sci. Food Agric.*, **36**, 1259.

Bartley, I. & Knee, M. (1982). The chemistry of textural changes in fruit during storage. *Food Chem.*, **9**, 47.

El-Zoghbi, M. S. (1989). Studies on pectin and other polysaccharides in mango and guava. PhD thesis, Faculty of Agriculture, Zagazig University, Egypt.

Gizis, E. J (1964). The isolation and characterizations of the pectic enzymes and the pectin substances of the north-west strawberry. PhD thesis, Oregon University, Covlis, OR, USA.

Hasegawa, S. & Smolensky, D. (1971). Cellulase in dates and its role in fruits softening. *J. Food Sci.*, **36**, 966.

Hintion, D. & Pressey, R. (1974). Cellulase activity in peaches during ripening. *J. Food Sci.*, **39**, 783.

Hobson, G. (1968). Cellulase activity during the maturation and ripening of tomato fruit. *J. Food Sci.*, **33**, 588.

Hobson, G. E. (19643). Pectinesterase in normal and abnormal tomato fruit. *Biochem. J.*, **86**, 356.

Honda, S., Nishimura, Y., Takahashi, M. & Chiba, H. (1982). A manual method for the spectrophotometric determination of reducing carbohydrates with 2-cyanoacetamide. *Anal. Biochem.*, **199**, 194.

Huber, D. J. (1983). The role of cell wall hydrolysis in fruit softening. In *Horticultural Reviews*, J. Janick. Avi Publishing Co., Westport, CT, USA, p. 619.

Huber, D. L. (1984). Strawberry fruit softening: the potential role of polyuronides and hemicelluloses. *J. Food Sci.*, **49**, 1310.

Hultin, H. & Levine, A. (1965). Pectin methylesterase in the ripening banana. *J. Food Sci.*, **30**, 917.

Jensen, E. & MacDonnel, L. (1945). Influence of methoxyl content of pectic substances on the activity of polygalacturonase. *Arch. Biochem.*, **8**, 97.

Mowlah, G. & Itoo, S. (1983). Changes in pectic components, ascorbic acid, pectic enzymes and cellulase activity in ripening and stored guava. *Nippon Shokuhin Kogyo Gakkaishi*, **30**, 454.

Mustafa, A., Harper, D. & Johnston, D. (1986). Biochemical changes during ripening of some Sudanese date varieties. *J. Sci. Food Agric.*, **37**, 43.

Nagel, C. & Patterson, M. (1967). Pectic enzymes and development of the pear. *J. Food Sci.*, **32**, 294.

Pressey, R. (1986). Polygalacturonases in higher plants. In *Chemistry and Function of Pectins*, ed. M. Fishman & J. Jen. American Chemical Society, Washington, DC, USA, p. 157.

Roe, B. & Bruemmer, J. (1981). Change in pectic substances and enzymes during ripening and storage of Keitt mangoes. *J. Food Sci.*, **46**, 186.

Simpson, B., Egyankor, K. & Martin, A. (1984). Extraction, purification and determination of pectin in tropical fruits. *J. Food Process. & Preserv.*, **2**, 63.

Sobotka, F. & Stelzig, D. (1974). *Plant Physiol.*, **53**, 759, *CF*: Recent advances in the biochemistry of fruits and vegetables, ed. J. Friend & M. Rhodes, Academic Press, London, p. 123.

Subramanyam, H., Krishnamurthy, S., Subhodra, N., Datal, V., Randhawa, G. & Cdacko, E. (1972). Studies on low temperature breakdown in mangos. *Ann. Rep. Cent. Food Techn. Res. Inst. 1967–1972.*

Tahir, M., Chaudry, M. & Malilk, A. (1975). Pectinesterase activity of orange during ripening. *Pak. J. Sci. Res.*, **27**, 59.

Updegraff, D. M. (1969). Semimicro determination of cellulose in biological materials. *Anal. Biochem.*, **32**, 120.

Van Soest, P. J. (1963). Use of detergents in the analysis of fibrous feeds. II: A rapid method for determination of fiber and lignin. *J. Assoc. Agric. Chem.*, **46**, 829.

Van Soest, P. J. & Wine, R. H. (1967). Use of detergents in the analysis of fibrous feeds. IV: Determination of plant cell wall constituents. *J. Assoc. Agric. Chem.*, **50**, 50.

Vas, K., Nedbalek, M., Scheffer, H. & Kovacs-Proszt, G. (1967). Methodological investigation on the determination of some pectin enzymes. *Freuchtsaft Ind.*, **12**, 164.

Voragen, A. G., Timmers, J., Linssen, J., Schols, H. & Pilnik, W. (1983). Methods of analysis for cell-wall polysaccharides of fruit and vegetables. *Z. Lebensm. Unters. Forsch.*, **177**, 251.

Yusof, S., Mohamed, S. & Abu Bakar, A. (1988). Effect of fruit maturity on the quality and acceptability of guava puree. *Food Chem.*, **30**, 45.