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Factors Influencing the Quality Characteristics of Frozen and Dehydrated Fruits and Vegetables

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David S. Reid.....

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Contents

Abstract

The effect of freezing or dehydration on the textural and other related properties of preserved fruits and vegetables has been investigated in peaches, green beans, carrots, and strawberries. Since it is believed that the pectic fraction is responsible for much of the non-turgor-related textural impression, the changes which take place in the pectic fraction as a result of either freezing or dehydration have been followed. Clearly defined changes take place in the composition of the pectic fractions, as a result of freezing or dehydration. The extent of change can be affected by appropriate treatment of the raw material prior to either freezing or drying. The most effective pretreatment is blanching. There is an observable effect of blanching on the subsequent changes which take place in the pectic fraction, during either freezing or dehydration processing.

Conditions which minimize subsequent degradative change in the pectin yield the highest quality products. The pectins are characterized as water soluble, chelator soluble, and protopectic fractions. All fractions change after processing. The extent of change is dependent on the individual tissue, and indeed the relative amount of the individual fractions is dependent on the tissue. Ion exchange chromatography of the pectic fractions has provided a finger print of the effects of processing. Underblanching and overblanching result in more marked degradative changes in the pectins. This is seen for both freezing and for drying. It is true for carrots, peaches, green beans and strawberries.

Pectin is confirmed as an important component contributing to texture, and in dehydrated tissue, to rehydratability. Blanching helps protect the pectin from degradation, and therefore is an important pretreatment which improves product quality.

Introduction

Since it is difficult to market fresh produce on a year round basis, preservation methods have a high economic significance. It is the purpose of preservation to yield a product of high quality, with an extended shelf life, so that market needs can easily be met. Freezing and dehydration are two important food preservation technologies, employed by both the fruit and vegetable industries. Both technologies are capable of producing high quality products, which possess a long shelf life, but these products are readily seen to differ from the original fresh tissue. Both freezing and dehydration produce a range of changes within a tissue subjected to either process. One of the most marked changes is associated with the texture of the final product. It was our objective in this project to study the textural changes resulting from freezing or drying, using instrumental methods. In order to explain the observed textural changes, our particular objective was to investigate the role of the pectic component of the tissue in the processes which led to textural alterations which could be detected by the instrumental methods.

In order to investigate the role of the pectic component, it was necessary to document the effect of a series of processing regimes on the quality, in particular the texturally related quality, of some processed tissues. By processed, in this context, is meant either frozen or dehydrated. In order for meaningful conclusions to be drawn as to the contributory role of

the pectins, it was particularly important also to examine the pectic material. Since it is not easily possible to examine pectin as it exists within any tissue, some form of extraction protocol had to be employed. The extracted material required fractionation, followed by examination of the individual fractions for evidence of change, presumed to be process related. It was necessary to assume that any changes in the pectin caused by the process would be evident on the extracted material. It had to be assumed that comparisons of the extracted materials would lead to conclusions which would also be true of the original tissue bound materials.

To extract pectins, an alcohol insoluble fraction of the tissue is prepared[1]. This should contain the pectic polymers, along with other cell wall components. This material, termed AIS, is then subjected to a fractionation scheme based on consecutive extractions by a series of aqueous solvent systems. Each solvent fraction, which has its own particular characteristics, can then be further studied by some form of chromatographic separation method. These fractions are termed WSP (water soluble pectin), CSP (chelator soluble pectin) and HSP (hydroxide soluble pectin, or protopectin)[2].

Methods

Freezing and dehydration have been carried out using standard procedures. For freezing, both fast and slow freezing have been employed where appropriate. Blanching has been performed by both the techniques of hot water blanching and steam

blanching. A range of blanching conditions have been used, which are identified where appropriate. In all cases, the range of conditions employed for blanching have included a light blanch, or no blanch, and a severe blanch, in excess of the treatment necessary to inactivate the major enzymes present. An extreme blanch would be expected to increase the extent of thermal degradative change to the tissue pectins. Figure 1 illustrates the general protocol used for much of the experimental processing.

Conventional analyses have been performed to characterize the tissues[3]. These analyses include enzyme assays where appropriate. Textural assessment has primarily used instrumental techniques. For example, one such technique is back extrusion on an Instron testing machine[4]. The force required to move a piston at a constant rate down into a cup of tissue material is recorded. At some point, the tissue extrudes back through the annulus between the piston and the wall of the cup. This "back-extrusion force" is recorded. Different tissues show characteristically different forces of back extrusion. Processing, and storage also results in change in the force. The higher the force recorded, the firmer the tissue. If the tissue softens, has there been a change in the materials of the cell wall complex?

The cell wall material has been prepared by precipitation of the alcohol insoluble solids using 75% alcohol. After washing and drying, fractionation of the AIS has involved extraction with water, to give a water soluble fraction, extraction with a

calcium chelator to give a chelator soluble fraction, followed by an alkaline solubilization technique to yield the protopectin fraction. The amount of pectin in each fraction is determined by an assay for uronic acid content[5], since the primary monomeric unit of pectin is galacturonic acid, and the other alcohol insoluble polymers of the cell wall complex do not contain any appreciable content of uronic acid. The fractions can then be further characterized by ion exchange chromatography, passing the materials through a suitable column in the appropriate solvent.

The tissues examined in this project included carrots, green beans, peaches and strawberries in the freezing study, and carrots and peaches in the dehydration study. The results are reported in terms of general observations on the effects of processing, including texturally related aspects of quality, as represented by the maximum observed back extrusion force. A lower value of back extrusion force for a given tissue is taken to reflect an increased softness of texture. The general observations of chaange in the tissues are then followed by specific studies of the effects of processing on the pectic fractions which can he prepared from the individual tissues. A report of some model experiments examining the efficiency of chelator extraction of pectin, when various chelators are employed, precedes the report of the tissue results.

Results and Discussion

As indicated, after preparation of the AIS, this material is fractionated to yield water soluble, chelator soluble, and protopectic fractions. Since some pectin in the cell wall complex may be associated with calcium, and hence be difficult to extract with water alone, a chelator may be used to destabilize this complex, and allow the pectin to be extracted into a water based solvent. As can be seen from figure 2, which reports a series of extractions on the AIS prepared from green beans, the efficiency of extraction is dependent on the individual chelator used. We have chosen to perform the extraction at a pH close to neutrality, to minimize other possible change affecting the pectic material, such as hydrolytic cleavage or beta elimination. As a result the oxalate ion, which is often used in fractionation schemes for pectic materials in cell walls, would appear to be an inappropriate choice for chelator. One reason for this is that calcium oxalate is insoluble, and may indeed trap pectin within the tissue matrix. The use of EDTA as chelator gives a much more effective extraction. This conclusion has also been reached in a similar investigation utilizing the AIS extracted from carrot. A manuscript describing part of this study has been prepared for publication. A copy of the draft is attached in an appendix to this report. For this study the chelator of choice has been EDTA, dissolved in a Tris buffer at pH7.

The results of the overall assessments of quality change in

processed tisues are summarized in table 2. All data have been compiled from draft manuscripts attached as appendices to this report. It is clear that some tissues yield better thawed or reconstituted products (after freezing or dehydration as appropriate) if a suitable blanching treatment has been utilized prior to the main process. The result of too little or too much blanching is to yield a product of poorer quality. The residual enzyme activity after typical blanches is also summarized in table 2 for carrots and beans. In some tissues, such as strawberries, blanching is an inappropriate pretreatment, since it will produce severe textural damage in the tissue. Here, too, appropriate pretreatments can yield higher quality products. In this case pretreatment with calcium results in a firmer texture. There is a clear change in texture as a result of freezing. This change is additional to any change produced by blanching. There is a slower alteration in texture which takes place during frozen storage.

In order to better understand some of the observations summarized in table 2, the patterns of change in the pectic fractions have to be examined. In tables 3 to 7, the results of the fractionation and subsequent analysis of the pectins after a variety of processing options are summarized. Clearly, processing results in some degradation of the pectic fraction of the cell wall matrix. The steps of degradation may be through protopectin to chelator soluble pectin to water soluble pectin to uronic acid oligomers and monomers. Loss of total pectins probably reflects degradation to small oligomers. The amounts of the individual

pectic fractions change through the influence of processing. Also, as can be seen from figures 3 and 4, the chromatographic profiles of these fractions change in characteristic ways. The effect of the processing on the pectins can be understood, at least qualitatively. Both thermal degradation (due to blanching or some other heat treatment), and degradation due to the action of enzymes, can be seen. Some loss of methylation is evident. Water soluble pectins can be lost in the drip. The molecular weight of the pectins is lowered by cleavage of the polymer chain.

From the results summarised in the tables, it is clear that there is a degradation in textural quality which accompanies the degradation in pectin. This is clearly seen as a reduction in the characteristic force for back-extrusion in the "back-extrusion cell". Some of this textural degradation may be due to ice crystal formation in frozen systems. The growth of ice crystals during frozen storage can be seen in the scanning electron micrographs of isothermally freeze fixed frozen green beans shown in figure 5. We do not have at present similar micrographs prepared from dried tissues, but figure 6 shows micrographs which illustrate the effect of freezing and blanching on carrots.

The detailed results of this study are contained in the draft manuscripts of the appendices.

Conclusion

Thermal processing results in some degradation of pectins. However, the degradation which occurs in unblanched tissues can be more severe. An adequate blanch helps minimize the degradative

change in pectins in both frozen processing, and dehydration processing, and is important to the maintenance of texture and quality. Individual reports from this study have made recommendations as to the optimum process to employ to minimize detrimental change to the pectic component of processed fruit and vegetable tissue.

References

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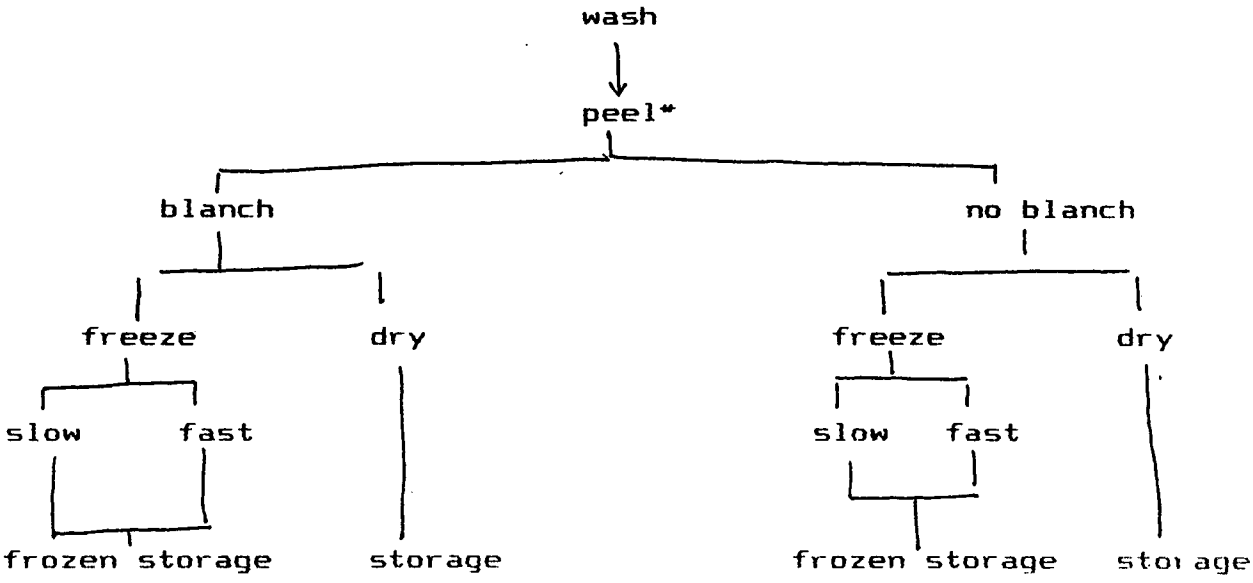
Figure 1

Schematic of processing protocol



* optional procedure

Table 1

Processing methods

Blanching:
(a) Water blanch. Used on produce to be dehydrated, at specified temperatures.
(b) Steam blanch. Used on product to be frozen. Tunnel steam blancher operated at temperature and with holding times specified.

Freezing:
(a) Fast freezing utilized a blast freezer with air temperature -45°C or less.
(b) Slow freezing used a plate freezer with plate temperature -20°C (Volcani) or a still air condition in a cold room with an air temperature of -20°C (UC Davis).

Dehydration:
Hot air drying, temperature and time specified.

Analytical methodologies are described in the draft manuscripts of the appendices.

Table 2

Effects of processing on quality characteristics of tissues

(a) Carrot.. general processing parameters

Treatment	yield	TSS	Vit C	AIS*
fresh	100%	8.2	7.4	3.0
peel	81	7.7	4.5	2.77
+2 min blanch	75.4	7.3	4.0	3.1
+4 min blanch	76	7.4	3.4	2.9

TSS Total soluble solids
water blanch at 99°C

(b) Carrot.. enzyme inactivation and texture

Blanch Time	Peroxidase Retention	Catalase Retention	PE Retn	Back-extrusion Force
0	100%	100%	100%	183kg
1	2.2	1.4	2.9	107
2	.13	1.6	.46	86
5	0	1.0	0	31.8

Steam blanch at 98°C

after 2 months frozen storage and thaw

				2mo	7mo
0	100	100	4.6	58.9	29.6
1	1.2	1.0	.89	48.6	23.5
2	0	.88	.15	35.	15.4
5	0	.81	0	20.8	8.0

PE Pectin esterase

(c) Peaches (water blanching and sulfuring)

Blanch time	Dry matter	AIS	Pectin	Firmness
0 min	17%	13.2%	510mg/100g	21.6newtons
5	15.2%	15.9%	454	15.5
15	14.2%	15.4%	349	7.8

after drying, result of pectin assay

blanch time min	total pectin mg/100g	protopectin mg/100g	rehydration weight
0	1780	450	277%
5	2628	731	330%
5*	2781	1135	347%
15	2428	512	294%
15*	2482	593	314%

* sample from second batch of peaches

(d) Green Beans

Blanch time	total solids	PO act	LIP act	PE act	BEF	Drip loss
0min	11.3%	100%	100%	100%	174kg	1.7%
1	10	3.5	1.8	0.5	165	2.9
2	10.5	-	2.6	0.3	145	2.8
3	10.8	-	1.9	0.4	118	1.3
4	10.2	-	2.1	0.8	127	1.2
5	10.2	-	1.9	0.7	69	0.4

PO peroxidase
LO lipoxygenase
PE pectin methylesterase
BEF back-extrusion force

Steam blanch at 98°C

Table 3

Characteristics of pectins extracted from differently processed materials. Amount of pectic fractions in the AIS prepared from different tissues.

TABLE

(a) Strawberry blast frozen -20 C storage
(variety Aiko)

	unfrozen	1 week	10 mo	10 mo(*)
WSP	48.6	44.5%	31.2%	47.2%
CSP	14.1	15.4	17.4	14.8
HSP	9.5	9.8	12.6	10.1
Sum	72.2	69.7	61.2	72.1

(*) AIS prepared directly from frozen tissue, with no drip loss occuring, unlike regular preparation.

(b) Strawberry slow frozen -12 C storage
(variety Pajaro)

	unfrozen	1 day	4 month
WSP	44.63%	46.98%	47.17%
CSP	24.80	19.48	18.43
HSP	24.05	23.83	21.86
Sum	93.48	89.79	87.46
RESIDUAL	15		

(c) Peach unblanched -20 C storage

	unfrozen	1 day	4 month
WSP	23.56%	26.28%	23.74%
CSP	8.86	9.27	11.82
HSP	36.09	40.52	52.68
Sum	68.51	76.07	88.24
RESIDUAL	22		

(d) Green bean 2m blanch slow frozen -12 C storage

	unfrozen	1 month
WSP	8.81%	8.32%
CSP	19.33	22.31
HSP	21.33	18.85
Sum	49.48	49.48
RESIDUAL	36	

tissues*.

Fraction	Strawberry	Peaches	Green beans
Total uronide	100%	100%	100%
WSP	44.6	23.6	8.8
GSP	24.8	8.9	19.3
HSP	24.1	36.1	21.3
SUM	93.5	68.6	49.4
Residue	15.3	21.6	35.4

*Unblanched unfrozen tissue for strawberry and peaches, and 2 min blanched at 212°F, unfrozen for green beans.

Pectic fractions of blast frozen peaches stored at -20

Fraction	Unfrozen		1 Day		4 Months	
	µg GA/µg CWAIS	%	µg GA/µg CWAIS	%	µg GA/µg CWAIS	%
Total	0.4415	100.0	0.4077	100.0	0.3321	100.0
WSP	0.1040	23.56	0.1071	26.27	0.0788	23.73
CSP	0.0391	8.86	0.0378	9.27	0.0393	11.83
HSP	0.1593	36.08	0.1652	40.52	0.1749	52.66
SUM	0.3024	68.50	0.3101	76.06	0.2930	88.23

Table 4

Neutral sugar contents of various pectin fractions. Analysis by the method of Albersheim et al.

Neutral sugar composition of Soluble Pectin in the A.I.S (mg/g dry matter).

Sugar	Control	%	Blanching	%	Blanching Dehydration	%	Dehydration	%
Rhamnose	0.09	0.8	0.13	1.1	0.11	0.9	0.09	0.9
Arabinose	0.53	5.0	0.72	6.1	0.81	7.1	0.66	7.2
Xylose	0.05	0.4	0.06	0.5	0.07	0.6	0.09	0.9
Mannose	0.59	5.1	0.26	2.2	0.33	2.9	0.23	2.5
Galactose	0.94	8.9	1.72	14.7	1.07	9.4	1.05	11.5
Glucose	0.23	2.1	1.06	9.0	0.74	6.5	0.68	7.4
Total Neutral Sugars	2.43	22.3	3.95	33.8	3.13	27.5	2.81	30.7
Galacturonic acid	8.11	77.7	7.72	66.2	8.24	72.5	6.30	69.3
Total sugars	10.54	100.0	11.67	100.0	11.37	100.0	9.11	100.0

The content of galacturonic acid in the pectic fractions as a result of the indicated treatments (mg/g dry n matter).

TREATMENTS	SOLUBLE PECTIN	%	CALCIUME PECTATE	%	PROTO-PECTIN	%	TOTAL G.A.
UNTREATED TISSUE	8.11	16.9	11.54	24.1	28.13	58.8	47.78
BLANCHED TISSUE	7.72	12.4	20.30	32.8	33.76	54.6	61.78
BLANCHED & DEHYDRATED TISSUE	8.24	16.8	19.03	38.8	21.73	44.3	49.00
DEHYDRATED TISSUE	6.30	35.9	4.11	23.4	7.11	40.5	17.52

Total carbohydrates in the A.I.S after various treatments (mg/g dry matter).

	Fraction						Total sugars	
	Soluble Pectin	%	Calcium Pectate	%	Proto-Pectin	%	Total	%
UNTREATED TISSUE								
NEUTRAL SUGARS	2.43	3.8	1.07	1.7	11.58	18.4	15.08	23.9
GALACTURONIC ACID	8.11	12.9	11.54	18.3	28.13	44.7	47.78	76.1
CARBOHYDRATES	10.54	16.7	12.61	20.0	39.71	63.1	62.86	100.0
BLANCHED TISSUE PH-6.3								
NEUTRAL SUGARS	3.95	5.0	2.94	3.7	9.75	12.4	16.64	21.2
GALACTURONIC ACID	7.72	9.8	20.30	25.8	33.76	43.0	61.78	78.8
CARBOHYDRATES	11.67	14.8	23.24	29.6	43.51	55.4	78.42	100.0
BLANCHED DEHYDRATED TISSUE								
NEUTRAL SUGARS	3.13	5.4	2.62	4.5	2.99	5.1	8.74	15.1
GALACTURONIC ACID	8.24	14.2	19.03	32.9	21.73	37.6	49.00	84.9
CARBOHYDRATES	11.37	19.6	21.65	37.4	24.72	42.8	57.71	100.0
DEHYDRATED TISSUE								
NEUTRAL SUGARS	2.80	11.8	0.64	2.1	2.57	10.9	6.01	25.5
GALACTURONIC ACID	6.30	26.7	4.11	17.4	7.11	30.2	17.52	74.5
CARBOHYDRATES	9.10	38.6	4.75	20.1	9.68	41.1	23.53	100.0

Table 5

Characteristics of the pectic fractions separated on DEAE sephadex columns. Typical separations are illustrated in figures 3 and 4.

Neutral sugars ratios from anion exchange chromatography fractions of strawberries WSP, still air frozen -12c storage.

sample	tot neutral sugars : tot uronides	Rhamnose	Fucose	Arabinose	Xylose	Mannose	Galactose	Glucose
unfrozen unbound fraction	0.24 : 1.0	4.5	2.20	18.0	19.6	6.8	35.4	13.4
unfrozen bound fraction	0.21 : 1.0	29.1	3.1	24.3	5.6	5.7	19.6	12.6
1 day storage unbound fraction	0.18 : 1.0	5.3	3.0	15.3	15.7	4.9	40.1	15.7
1 day storage bound fraction	0.32 : 1.0	31.2	-	21.5	8.6	8.5	17.9	12.4
4.5 month unbound fraction	0.14 : 1.0	5.6	3.5	17.0	16.9	3.7	39.0	14.3
4.5 month bound fraction	0.13 : 1.0	30.1	1.2	20.7	3.9	2.1	19.5	21.6

The content of total galacturonic acids and neutral heated sugars after separation on DEAE column in the calcium pectate fracture of untreated and heated carrot tissue.

The sugar content is expressed in micrograms and as a percent of total pectic substances.

Treatment	Sugar Content (ug) Fraction No. A	%	B	%	C	%	Total Sugars ug	%
Untreated tissue								
Neutral sugars	680	3.1	1260	5.8	180	0.8	2120	9.7
Galacturonic acids	761	3.5	18720	85.6	262	1.2	19743	90.3
Pectic substances	1441	6.6	19980	91.4	442	2.0	21863	100.0
Blanched tissue								
Neutral sugars	1796	6.8	3915	14.8	24	0.1	5735	21.7
Galacturonic acids	5184	19.6	14616	55.2	954	3.6	20754	78.3
Pectic substances	6980	26.4	18531	70.0	978	3.7	26489	100.0

The ratio between neutral sugars and rhamnose in the soluble pectin fraction of untreated and heated carrot tissue.

Neutral sugars Composition	Untreated tissue Fraction B_1	B_2	B_3	Heated tissue Fraction B	C
Arabinose	10.0	6.9	5.0	2.9	1.9
Mannose	0.5	1.7	1.0	1.2	0.9
Galactose	16.4	9.2	6.0	4.7	3.2

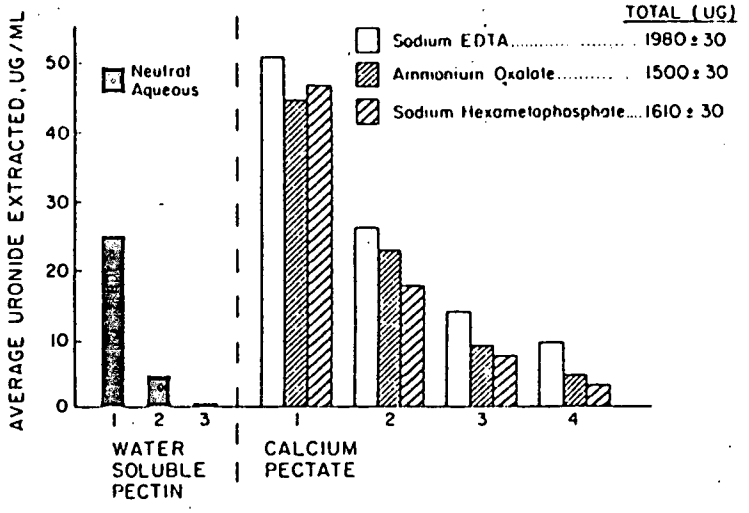
Table 6 Comparison of neutral sugar content and degree of esterification of WSP from three different tissues*.

Commodity	Total neutral sugars: total uronides	Percent DE
Strawberry	0.25 : 1.0	47.3
Peaches	0.37 : 1.0	80.1
Green beans**	0.41 : 1.0	0.0

Table 7 Percent degree of esterification of WSP from still air frozen strawberries -12°C storage.

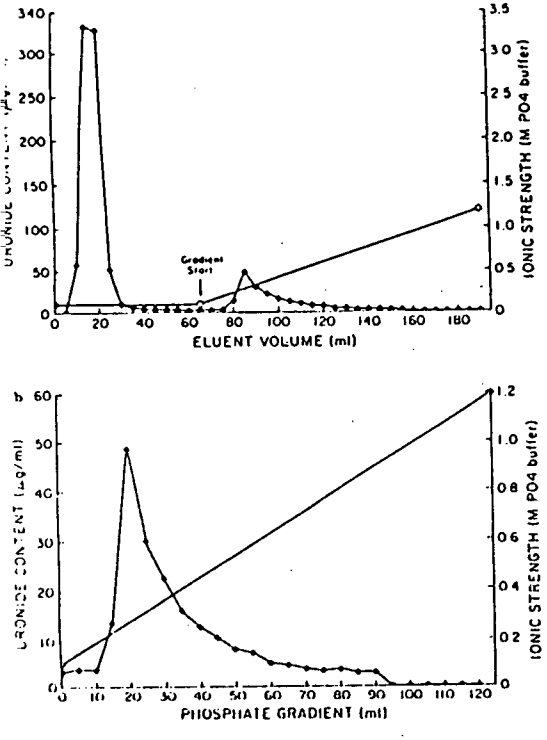
Sample	Percent D.E.
Unfrozen strawberries	
total WSP	47.25
unbound fraction	93.66
1 day storage	
total WSP	78.15
unbound fraction	86.25
4.5 month storage	
total WSP	43.91
unbound fraction	91.43

Figure 2. Effect of chelator on extraction of "calcium bound" pectin.

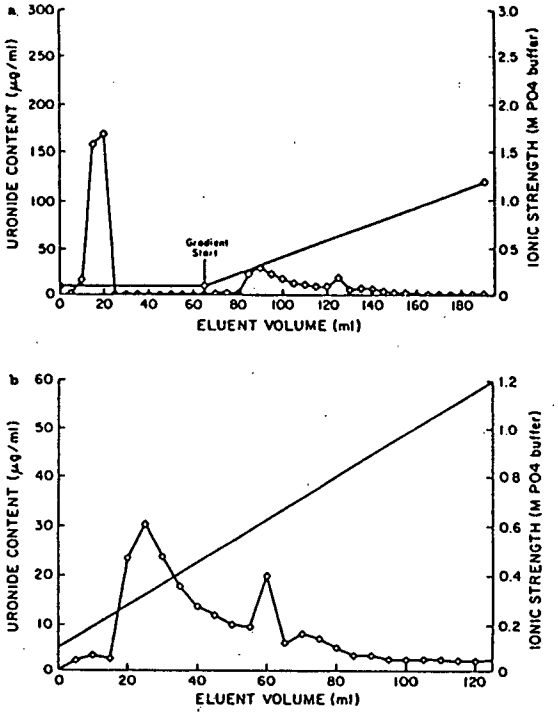


Extraction performance of different chelators at pH7 in AIS.

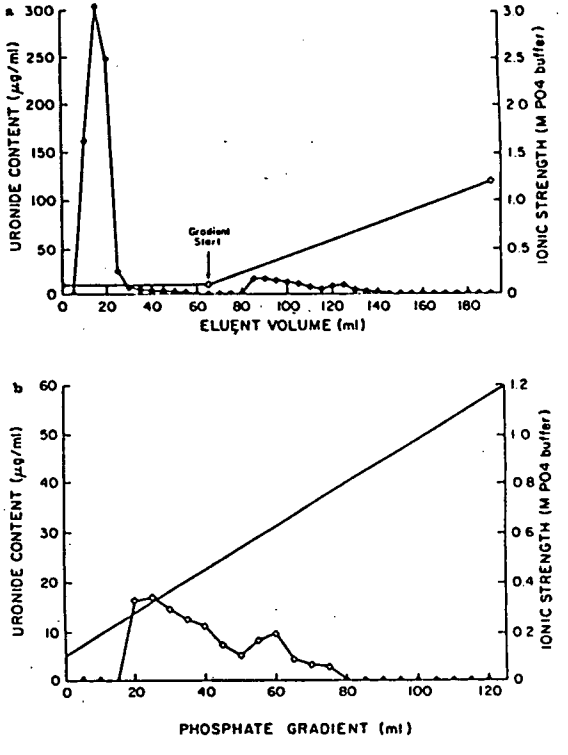
Figure 3. Column chromatographic fractionation of pectins derived from strawberries, as a function of time of storage.



. DEAE fractionation of WSP from 4.5 month frozen trawberries.
a) complete fractionation profile
b) elution of column bound material

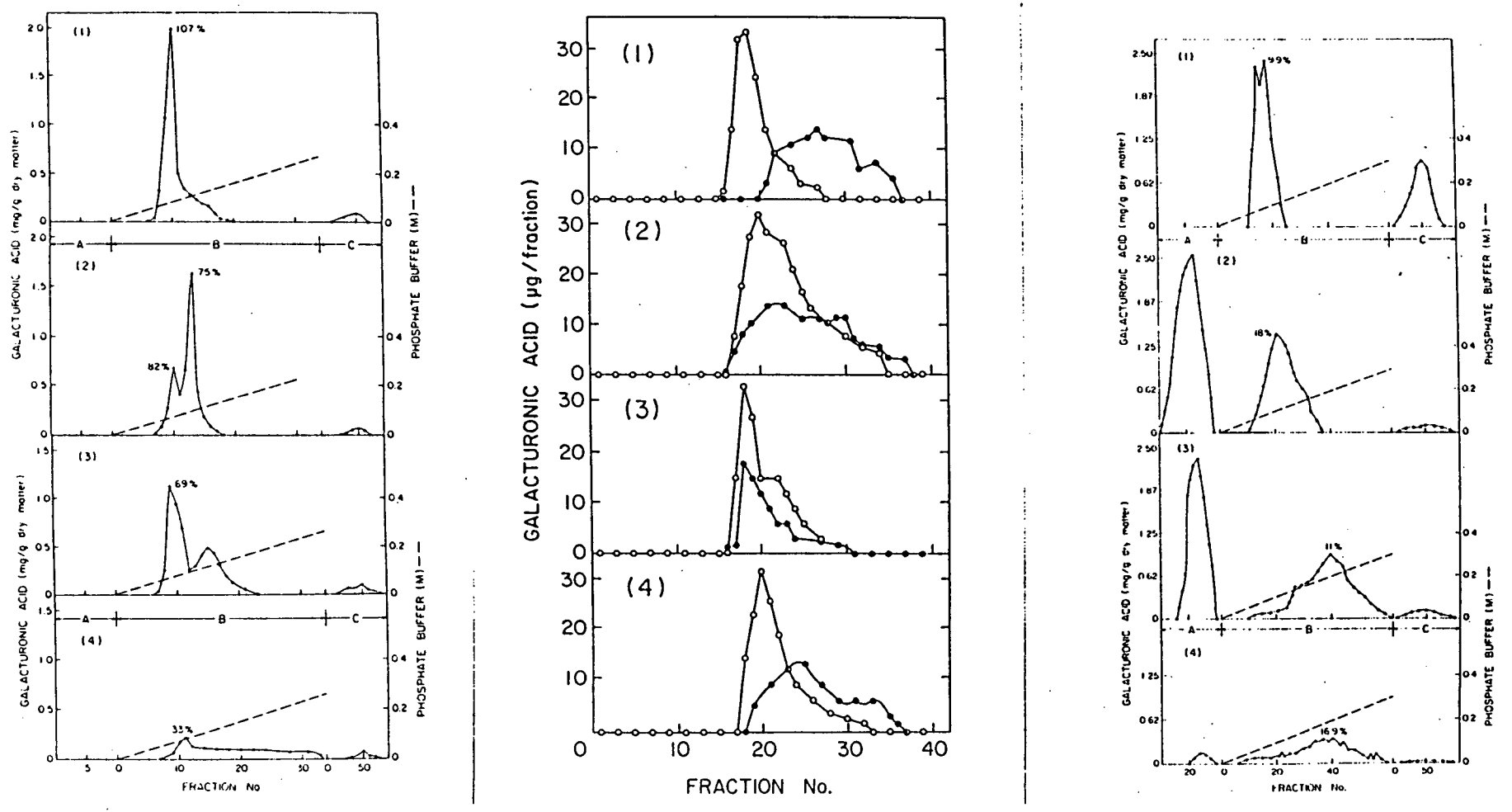


DEAE fractionation of WSP from unfrozen strawberries.
(a) complete fractionation profile
(b) elution of column bound material



DEAE fractionation of WSP from 1 day frozen strawberries.
(a) complete fractionation profile
(b) elution of column bound material

Figure 4. Column chromatographic fractionation of pectins extracted from processed carrots.



Description of cooperation.

The research performed under this grant, and summarized in this report, was carried out jointly in Israel at the Volcani Institute, and in California, at UC Davis. The bulk of the work relating to dehydration was performed at the Volcani, under the direction of A.Levi and N Benshalom. The freezing work was primarily performed in Davis, under the direction of D. Reid and B. Luh. Some tissues were studied in both places. The methods of pectin assay were jointly developed, and results were shared where practicable. The principal investigators met twice during the project to compare progress, and plan future work. One meeting was at the Volcani Institute, and the other at UC Davis. A series of joint publications are in preparation to report the results of the investigations. Draft manuscripts are attached where they are sufficiently developed. Some of these manuscripts have already been submitted for publication, others are under revision prior to submission. The results of the two segments of the study are in essential agreement as to the importance of pectin to textural quality, and to the extent of degradation of pectin which occurs as a consequence of processing.

Appendices

Draft manuscripts currently being prepared for publication are contained in this appendix. More detailed discussion of results is given. The manuscripts included are:

1. Effects of Freezing and Frozen Storage on characteristics of Pectin extracted from Cell Walls. Ap1-9.
2. Physicochemical changes occurring in green bean tissue during blanching, freezing and frozen storage. Ap10-24.
3. A comparison of calcium bound uronide extraction from green bean cell wall material using different chelating agents. Ap25-35
4. Physicochemical changes occurring in strawberry tissue due too freezing and frozen storage. Ap36-46
5. Enzyme, texture and quality changes in diced carrots during blanching, freezing and frozen storage. Ap47-67
6. Degradation of pectic substances in carrots by heat treatment. Ap69-86
7. Effect of blanching and drying on pectic constituents and related characteristics of dehydrated peaches. Ap87- 113.

16

Effects of Freezing and Frozen Storage on the Characteristics of Pectin Extracted from Cell Walls

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The effect of freezing and frozen storage on the texture of green beans peaches and strawberries has been assessed. The textural changes result from more than solely turgor pressure loss. The pectic component of the cell wall complex has been extracted and characterized as a function of the freezing method and frozen storage conditions. Changes in this pectic fraction could account for some of the textural alteration observed.

Texture is an important attribute of the eating quality of fruits and vegetables. An important aspect of texture is firmness. Texture or firmness result from a variety of contributions. Amongst these contributions are the turgor of an intact cell, and the strength of individual cell walls. It has often been remarked that a major textural consequence of freezing and frozen storage on fruits and vegetables is a loss of tissue firmness. Whilst it is known that freezing causes severe damage to the membranes of cells, and is therefore responsible for a loss of turgor, it is less clear whether there is a contribution to loss of firmness from the cell wall component. It should be remembered here that the softening of tissues during ripening has been shown to be largely a result of changes in the cell wall. In particular, it has been demonstrated that there is a correlation between the pectic component of cell walls, and the tissue firmness. In the study described here we wish to investigate whether some of the softening of plant tissues which accompanies freezing and frozen storage is related to changes in the cell wall, in particular whether there are related changes in the pectic fraction of the cell wall. This requires that we in some way assess texture, and that we also quantify and characterize the pectic materials of the tissue.

Experimental Procedures

In order to assess texture, we have employed a back extrusion cell accessory on an Instron Universal Testing Machine. The cell,

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illustrated in figure 1, is modified from a design described by Bourne and Moyer(1968). The Instron cross-head is moved at 100 mm/min. A sample of material, contained in the cup, is compressed as the plunger comes down. At length, the material extrudes back through the annulus between plunger and cup wall. A typical force-distance curve is shown. The rise reflects the compression, and t plateau of the back extrusion. We use the plateau force as a measure of firmness. Ten replicate measurements are performed. Repeatability is better than 4%.

In order to quantify and characterize the pectin component of the cell, we must use some procedure to prepare a stable cell wall fraction, and then follow some predetermined extraction scheme whi allows for the separation of pectic materials of different characteristics. The overall amount of pectin in any situation is then determined by assaying for uronic acids. The extractions of the pectic fractions need not be exhaustive, but must be repeatabl since we wish to follow the changes which may take place in the fractions consequent upon freezing and frozen storage. The separation procedures we have employed are as follows. We have chosen alcoholic extraction of macerated thawed tissue as a method for the preparation of a cell wall material which will be stable, and suited to storage prior to further assay. Cell wall material was prepared according to a method modified from that of Ahmed and Labavitch (1977) by adding washing steps using chloroform/ methano and acetone. A flow chart of the procedure is shown in figure 2. The wash steps were included as we found that material extracted using only alcohol tended to form a glassy, intractible material o drying. This was particularly true of strawberries. The material for extraction generally has been drained during the thawing procedure, and it is this drained, thawed material which is macerated. The drained liquid is collected separately, and identified as drip.

The extraction of pectic fractions from the cell wall materi follows the traditional logic. First a water soluble fraction is prepared. This is obtained by taking 100mg of cell wall material and shaking it vigorously with 20ml of water. After standing for minutes, the supernatant fluid is separated by centrifuge, and the solid material is treated with more water. The treatment is performed four times, and the supernatant fluids combined as the water - soluble pectic fraction (WSP). The residual solid is the used to prepare the chelator soluble fraction (CSP). A similar procedure is used to that already described, excepting that the solvent is 0.1M EDTA in 0.1M Tris, pH7. A final fraction, (HSP), prepared by subjecting the residue of step 2 to dilute sodium hydroxide as solvent. Again, four stage extraction is performed. It should be noted that these procedures do not extract all the material of the cell wall preparation which contains uronic acid residues. The residue of step 3 is found still to contain uronic acid. This can only be brought into solution by using more extre methods for solubilization. Replicate extractions are performed.

The materials obtained are analysed for pectin content by uronic acid assay. The method of Blumenkrantz and Asboe-Hansen (1973) is employed. Further characterization of the pectic mater included assay for neutral sugar composition by the alditol aceta method of Albersheim et al (1967), which entails derivitization,

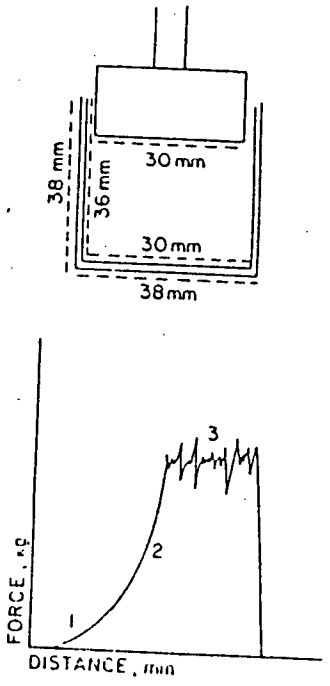

Figure 1. The back extrusion cell.

AIS Preparation/Purification

Ethanol: Chloroform-Methanol: Acetone Procedure

1. Wash and trim raw material.
2. Weight 100 g raw material and place in Waring blender with 4 mls 70% ethanol.
3. Homogenize for 1 minute.
4. Transfer slurry to 50 ml round-bottom centrifuge tubes and c
5. Centrifuge at 19,000 x g (12,500 rpm, ss-34 rotor) for 10 minutes.
6. Discard supernatant and transfer pelleted material to a cour sintered glass funnel.
7. Breakup pelleted material using a metal spatula.
8. Wash with 2 x 100 ml volumes 70% ethanol (applying vacuum aft thorough mixing of the solids and solvent).
9. Wash with 3 x 100 ml volumes chloroform-methanol (1:1 v/v).
10. Wash with 3 x 100 ml volumes acetone.
11. Air dry residue.

Figure 2. Flow chart of extraction procedure.

determination of the acetylated neutral sugars, in our case by capillary GC. In some cases, the extracts were separated further using column chromatography, the column containing DEAE sephadex, and elution being by an ionic gradient. Some samples were then subjected to gel filtration on a 1.1 x 60cm Biogel P100 column, in a 40mM Acetate, 40 mM EDTA, 50 mM NaCl buffer, pH 6.5, in order to estimate their approximate molecular weight profiles.

Materials

In order to study the effect of freezing on texture, and texturally related parameters, we have chosen three tissue systems. Green beans, strawberries and peaches. Green beens were of the Gallatin variety. Peaches were of the variety Halford. Two varieties of strawberry were studied, Aiko and Pajaro. All were obtained as fresh material, and processed in our pilot plant. Blanching, where required, utilized a steam blancher. Fast freezing used a Conrad freezer with an air blast temperature of -70 C. Products were frozen unwrapped in a single layer on open mesh trays. Immediately after freezing they were sealed into bags for storage. Slow freezing was in still air in a cold room. In this case the materials were sealed into bags prior to freezing.

Results

Texture is an important attribute of the three tissues chosen for study. Since blanching is an appropriate pretreatment to freezing in some cases, we might first ask the question " What is the effect of blanching on texture?" As blanching is a partial cooking process, not surprisingly, tissue softening occurs. We have taken samples of green beans and blanched them for increasing times at 100 C. Not surprisingly, measurement of the back extrusion force shows increasing softening with blanch time. At the same time, the blanch liquor has been assayed for pectin content, determined as uronic acid residues. The pectin released from the tissue as a consequence of blanching has been computed. Figure 3 shows the relationship between texture and released pectin. Clearly, the loss in texture is accompanied by a release of pectin. Another factor in the softening, of course, is the loss in turgor which accompanies the thermal destruction of the integrity of the cell membranes. What happens if we freeze the green beans?

As can be seen from figures 4 and 5, the result of freezing is a reduction in back extrusion force, whether the unfrozen material be blanched or not. The greatest reduction in back extrusion force as a consequence of freezing is seen for unblanched tissue, and presumably reflects in large measure the loss in turgor. However, loss of turgor is not the sole cause of the reduction in back extrusion force. Consider...blanching, too, destroys turgor, and yet the reduction in back extrusion force as compared to fresh tissue is greater for unblanched, frozen tissue than for blanched, unfrozen tissue. Also, there is a reduction in back extrusion force in blanched tissue due to subsequent freezing and thawing. This reduction is more marked, relative to the initial back extrusion force of the blanched tissue, for the more extensive blanches. Since it is unlikely that the turgor contribution can account for

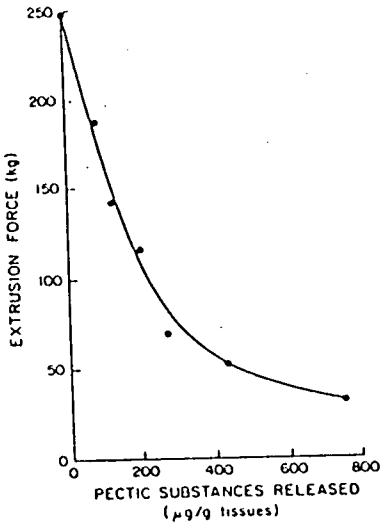

Figure 3. Relationship between texture and pectin release during blanching.

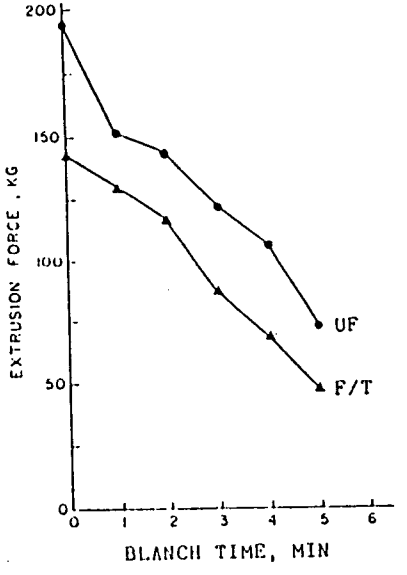

Figure 4. Effect of blanching and freezing on texture. UF refers to tissue which is not frozen after the blanch treatment, F/T to tissue which has undergone a freeze-thaw cycle after the blanch treatment.

this observation, it would appear that this loss in firmness is due to the cell wall contribution. Further, there is a continued decrease in tissue firmness as a consequence of extended frozen storage. It is therefore appropriate to follow the changes which take place in cell wall components as a function of freezing and frozen storage, and try to correlate these with observations relating to texture, tissue structure, etc. As already indicated, we have chosen to focus on the pectic fraction, since it is amenable to extraction and fractionation by reasonably simple methods. EDTA was chosen as chelator in part because we wished to perform our extractions under as mild conditions as possible, ie at room temperature, and close to neutrality, in order to minimize chemical change in the pectic fractions during their extraction. As can be seen from figure 6, the extraction efficiency of other commonly used chelators appears to be less than that of EDTA. This observation is discussed further by Reid and Carr (paper in preparation) and in Carr(1964).

Our results are best presented in stages. First we should consider the pectic composition of the unprocessed raw material. Then we should consider the changes which take place in the gross fractions as a consequence of processing and storage. To do this we need only to determine the amounts of pectin in each fraction. After this we should consider whether there have been any compositional changes in the pectin. This is best determined by using column techniques to fractionate the three categories of pectic material, and also by further analysing the pectins. If any changes are taking place as a consequence of processing, we would expect to see them reflected in at least some of these results.

Table 1 summarizes the data relating to the uronic acid content of individual pectic fractions from the three tissues both before and after processing. The data for the pectic fractions from unfrozen tissue include an assay for the uronic acid content of the residual material after the extraction of the three soluble fractions in order to confirm that we can account for all the uronic acid residues. The total uronic acid content of the original cell wall material in all cases defines 100%. As table 1 shows, the distributions of pectic fractions from the three tissues are very different. Also, the residual uronic acid content of the extracted cell wall material varies for these tissues. However, the residual assay indicates that we are accounting for most of the uronic acid containing material. The fractionation procedure extracts about 90% of the available uronic acid in Pajaro strawberry cell wall preparation, yet Aiko has an extraction of 70%. Only about 50% of the uronic acid in green bean cell wall preparation is extracted. The extraction efficiency for peaches increases during storage, suggesting that there is some change taking place.

Figure 7 illustrates the change in uronide content of the fractions obtained from the cell wall material as a function of the storage time at -20 C for the strawberry variety, Aiko. It is clear that the major change is in the uronic acid content of the water soluble fraction. This might be expected, if uronic acid containing wall components are constituents of the material lost as drip. In table 1a we compare the cell wall fractions obtained by homogenizing thawed tissue, from which the drip has been lost, and frozen tissue, which has lost no material. There is a loss of uronide in the

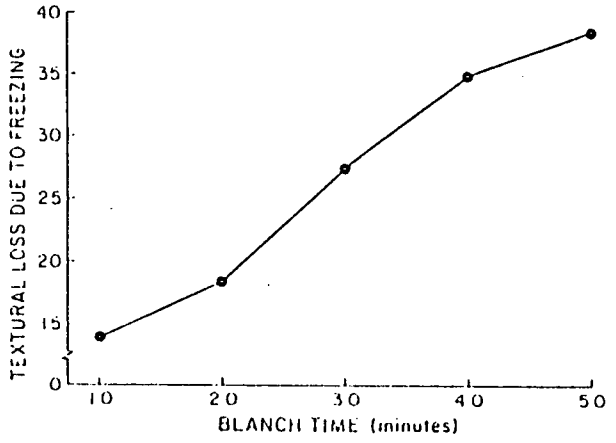

Figure 5. Texture loss due to freezing post blanch, calculated as the ratio of the loss in back extrusion force consequent upo freezing to the back extrusion force after blanching but prior to freezing.

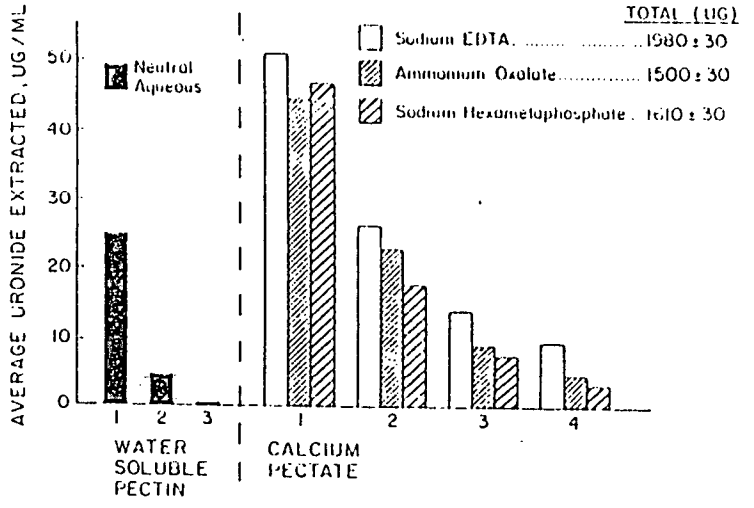

Figure 6. Extraction performance of different chelators at pH7 in AIS.

TABLE 1

(a) Strawberry blast frozen -20 C storage (variety Aiko)

	unfrozen	1 week	10 mo	10 mo(*)
WSP	48.6	44.5%	31.2%	47.2%
CSP	14.1	15.4	17.4	14.8
HSP	9.5	9.8	12.6	10.1
Sum	72.2	69.7	61.2	72.1

(*) AIS prepared directly from frozen tissue, with no drip loss occuring, unlike regular preparation.

(b) Strawberry slow frozen -12 C storage (variety Pajaro)

	unfrozen	1 day	4 month
WSP	44.63%	46.98%	47.17%
CSP	24.80	19.48	18.43
HSP	24.05	23.83	21.86
Sum	93.48	89.79	87.46
RESIDUAL	15		

(c) Peach unblanched -20 C storage

	unfrozen	1 day	4 month
WSP	23.56%	26.28%	23.74%
CSP	8.86	9.27	11.82
HSP	36.09	40.52	52.68
Sum	68.51	76.07	88.24
RESIDUAL	22		

(d) Green bean 2m blanch slow frozen -12 C storage

	unfrozen	1 month
WSP	8.81%	8.32%
CSP	19.33	22.31
HSP	21.33	18.85
Sum	49.48	49.48
RESIDUAL	36	

thawed tissue. Table 2 shows an analysis of cell wall uronide fr thawed tissue, together with the uronide assayed in the drip collected during the thaw. Soluble uronide is indeed lost in the drip, leaving the cell wall material depleted of material which would otherwise be included in the water soluble fraction.

Figure 8 shows that there is a continuous loss of texture in strawberries during frozen storage. This is paralled by the loss soluble pectin. It still remains to be determined whether, and i what manner, the pectin of the three fractions is changing in character. Given that the most dramatic loss in pectic component strawberries is in the WSP, figure 7, we have further characteriz this fraction by subjecting it to fractionation using a DEAE sephadex column eluted by an increasing ionic gradient. An amoun of solution containing about 7mg uronic acid is placed on the column, and eluted initially with 0.1M phosphate buffer, pH6.9. Once no more uronic acid material is found to elute, the gradient started, the final eluant being 1.2M phosphate buffer, pH6.9. Th remaining uronic acid on the column elutes in characteristic fashion. As can be seen from figures 9-11 there is a change in t elution characteristic of the WSP material as a function of stora time. We consider in particular the column bound material, that which does not elute until the ionic gradient is applied. These elution patterns are seen in figures 9b, 10b and 11b. The origin elution patterns for the column bound material show two peaks, at about 0.3 to 0.4 M ionic strength and at about 0.6M ionic strength. As a result of storage, the second peak decreases in size, whilst the initial peak remains unchanged. The fraction lo is the more acidic material, and probably represents the primary rhamno-galacturonan backbone, as indicated by the significantly higher proportion of rhamnose found in DEAE column bound material (table 3). Recent observations indicate that this rhamnose-rich uronide material is more slowly extracted from the AIS by water t is the material which elutes in the void volume of the DEAE column. To throw further light on the changes in this fraction, have performed gel filtration analyses to obtain preliminary indications of molecular weights. WSP materials collected from unfrozen, 1day frozen and 4.5month frozen strawberry samples all voided a P100 column, indicating molecular weights in excess of 100,000, based on elution of globular proteins of known size. Interestingly, material precipitated from the drip loss fraction with ethanol also displayed a major high molecular weight peak, w in addition about 20% of the total uronide containing material eluting with the totally included volume of the column. This wou correspond to a molecular weight around 10,000. The pectic mater in the drip is a significant part of the pectin lost from the cel wall in long term storage (table 2). Since, during frozen storag the drip loss increases, the nature of the uronide in the drip, a its change with time of storage, warrants further study. It woul be useful to know how the proportions of the high and low molecul weight fractions vary with time of storage.

Conclusions

Texture is affected by freezing in ways over and above the effect freezing on turgor. This suggests that there is a clear

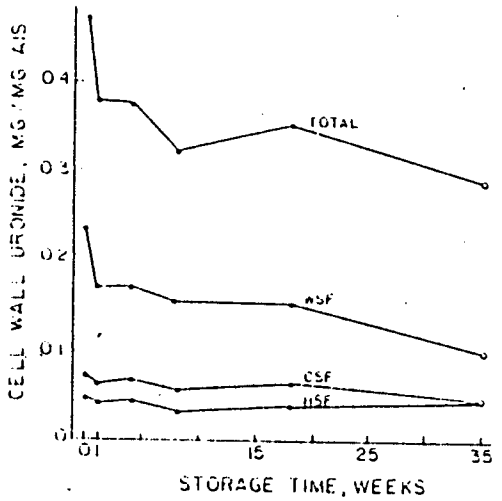

Figure 7. Uronide content of AIS fractions of Alko strawberries after different frozen storage times. WSF represents water soluble fraction, CSF chelator soluble fraction and HSF alkali soluble fraction.

Table 2
Strawberry drip analysis

Yield of uronide from 100g of fresh berry, frozen stored at -20 C for 9mo, then thawed for 90 minutes at room temperature.

	Berry	Drip
Total fresh wt	78.4	21.7
ug uronide/ ug AIS	0.218	0.106
Uronide in fraction (g)	0.417	0.040

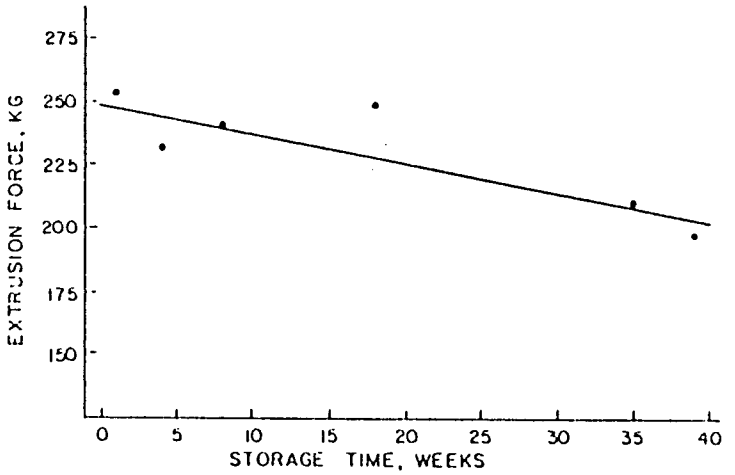

Figure 8. Texture loss in frozen storage of strawberries.

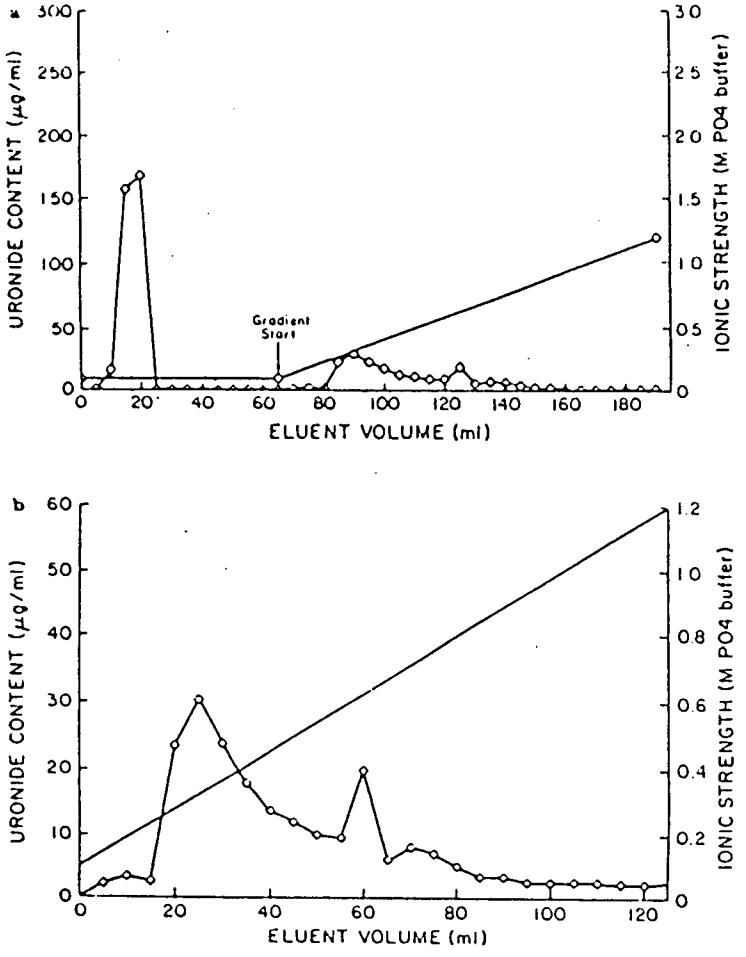

Figure 9. DEAE fractionation of WSP from unfrozen strawberries.
(a) complete fractionation profile
(b) elution of column bound material

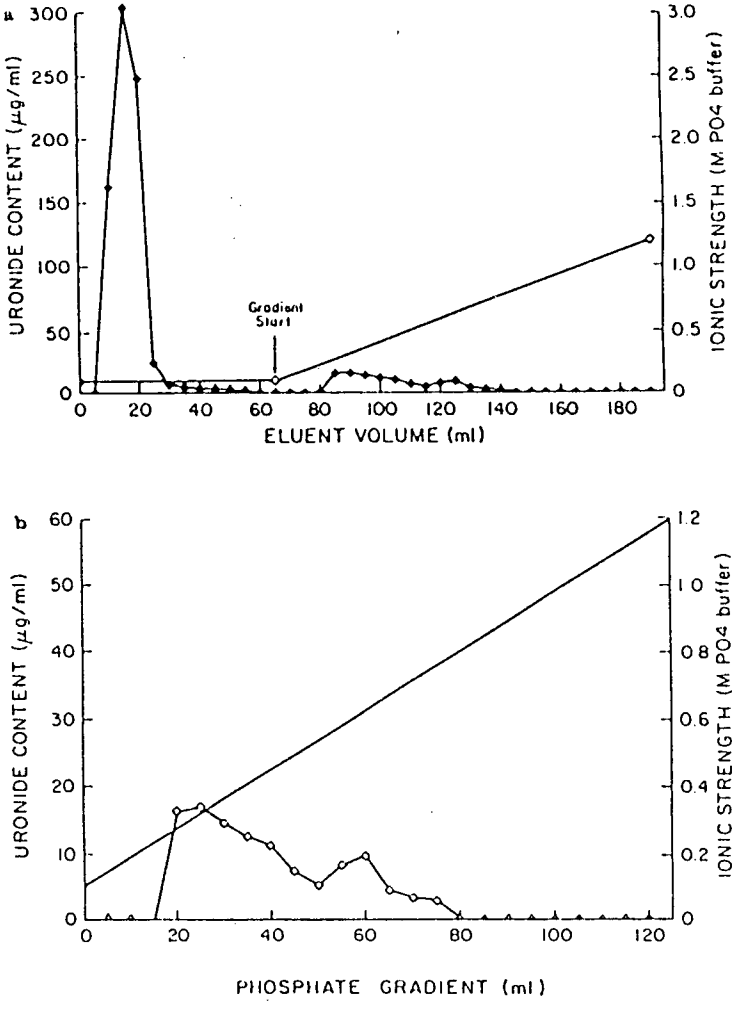

Figure 10. DEAE fractionation of WSP from 1 day frozen strawberries.
(a) complete fractionation profile
(b) elution of column bound material

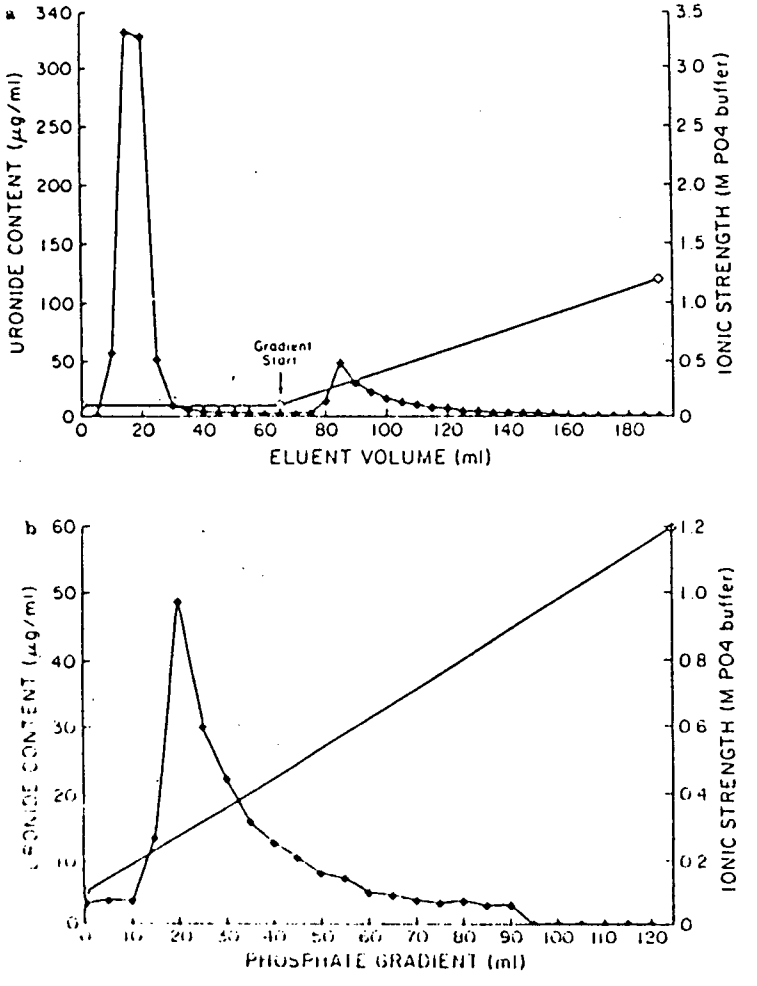

Figure 11. DEAE fractionation of WSP from 4.5 month frozen strawberries.
(a) complete fractionation profile
(b) elution of column bound material

Table 3. Neutral sugars ratios from anion exchange chromatography fractions of strawberries WSP, still air frozen -12c storage.

sample tot neutral sugars : tot uronides	Rhamnose	Fucose	Arabinose	Xylose	Mannose	Galactose	Glucose
unfrozen 0.24 : 1.0 unbound fraction	4.5	2.20	18.0	19.6	6.8	35.4	13.4
unfrozen 0.21 : 1.0 bound fraction	29.1	3.1	24.3	5.6	5.7	19.6	12.6
1 day storage 0.18 : 1.0 unbound fraction	5.3	3.0	15.3	15.7	4.9	40.1	15.7
1 day storage 0.32 : 1.0 bound fraction	31.2	-	21.5	8.6	8.5	17.9	12.4
4.5 month 0.14 : 1.0 unbound fraction	5.6	3.5	17.0	16.9	3.7	39.0	14.3
4.5 month 0.13 : 1.0 bound fraction	30.1	1.2	20.7	3.9	2.1	19.5	21.6

contribution to texture from the cell wall material, a conclusion supported by abundant material following the changes in cell wall pectins during fruit ripening. Pectin is also an important contributer to the texture of tissues which have been frozen. Changes are seen in the pectic material as a consequence of freezing and frozen storage.

In strawberries, the WSP fraction, which we assume to be the fraction most loosely associated with the cell wall, shows the most dramatic change. There is a decrease in amount, paralleling a decrease in firmness. The compositional studies of the fraction suggest that the changes include some associated with the pectin rhamno-galacturonan backbone. The chemical change in the backbone is not yet clearly defined. Changes in the sugar distribution and in the uronic acid to neutral sugar ratios are not in evidence as we analyse the deae bound fractions from fruits at progressively longer storage. The molecular weight remains above the cut-off for a P100 column.

Further studies are in progress. These include investigation of changes in the degree of methyl esterification of the fractions. The composition of the other fractions is being determined. The uronide fraction of drip is being analysed. The effect of adding calcium, which should interact with pectin, to strawberries prior to freezing them is also being assessed. Initial data indicate that calcium alters the relative amount of pectin extractable into the different fractions, and also alters the back extrusion force, and the nature of the time dependence of texture on frozen storage.

Acknowledgments

This research was supported by Grant No US-452-81 from BARD-The United States-Israel Binational Agricultural Research and Development Fund.

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17

Pectin Methylation Changes and Calcium Ion Effects on the Texture of Fresh, Fermented, and Acidified Cucumbers

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The commercial use of calcium ion in cucumber pickle products has stimulated efforts to better understand the mechanisms by which calcium affects cucumber texture. Recent results suggest that a high level of pectin methylation has little effect upon the ability of calcium to maintain the firmness of acidified cucumber tissue, while in fermented cucumbers maintenance of a minimum degree of methylation may be important to firmness retention. Efforts are being made to measure calcium binding characteristics in cucumber tissue and to obtain estimates of the effectiveness of calcium ion in inhibiting tissue degradation by pectolytic enzymes.

The ability of calcium ions to act as a firming agent in processed fruits and vegetables has been the subject of many studies over the years (1). Among the commodities in which calcium has been observed to cause firming are: snap beans (2), tomatoes (3), apples (4), carrots (5), apricots (6), and jalapeno peppers (7). Cucumbers are commercially preserved by fermentation (processed pickles), acidification and pasteurization (fresh-pack pickles) and refrigeration of mildly acidified fruit (refrigerated dills). Calcium ion has been found to be effective as a firming agent in all three types of products. It is now being used in most commercial cucumber pickle products. Investigations of the structure of cucumber cell walls and the interaction of calcium with the cell walls have followed the practical application of calcium.

There have been major advances in our understanding of the structure of plant cell walls over the past 15 years (8). However, it has not proven to be an easy task to explain specific textural changes which occur during ripening or processing of fruits and vegetables in terms of changes in the structures of cell wall polymers (9). This is perhaps not surprising since the detailed structures of cell wall polymers are proving to be very complex

TITLE:

PHYSICOCHEMICAL CHANGES OCCURRING IN GREEN BEAN (GENUS SPECIES) TISSUE DURING BLANCHING, FREEZING, AND FROZEN STORAGE

ABSTRACT:

The heat-induced softening that occurs during the blanching of green bean tissue was characterized. Heat treatment of green bean tissue caused a softening of the tissue as measured by an Instron back extrusion technique. This softening was accompanied by an increase in the amount of water soluble uronide material within the green bean tissue and additional water soluble uronide leached into the blanching medium. Freezing and thawing of the green bean tissue caused additional textural alterations and these were quantified for several different blanching treatments. Long term frozen storage of blanched and unblanched green bean tissue did not show any significant changes in product textural characteristics through 35 weeks of storage at $-20^{o}C$.

INTRODUCTION:

The enzymes of most vegetables and some fruits are often inactivated prior to freezing usually by a heat treatment or "blanch". Blanching causes a variety of changes within the tissue, not all of which are desirable.

The heating of fruit tissue often causes gross loss of textural crispness due to cell membrane damage, whereas many vegetables retain their frimness after heat treatment because they possess relatively thick cell walls. Parenchyma cells in the outer portion of the green bean pod would provide this type of textural effect (Brown, 1977). The thermally-induced softening process leaves a cell wall structure that remains essentially intact and continuous as can be seen by microscopic examination of heated tissue. In a review of fruit and vegetable texture, Brown (1977) also stated that cooking does not break green bean cell walls, though it does soften tissue.

The thinning of cell walls due to blanching of green beans is associated with the solubilization and loss of middle lamellar pectic substances. Mohr (1974) showed this cell wall thinning in various micrographs. Reeve (1970) also stated that all parenchymous fruits and vegetables undergo pectic changes during heating. He attributed their resulting loss of firmness to the formation of soluble pectins.

Hughes et al. (1975) found the loss of compressive strength of potato tissue during cooking to be related to the release of pectic substances into the cooking liquor. They found that starch release was not related to this loss of compressive strength. Further experimentation by these researchers showed that calcium chloride addition to the cooking medium reversed both compressive strength loss and increased pectin retention either due to a calcium-binding effect or to a drop in pH.

Albersheim and coworkers (1960) showed that heat treatment of model pectin solutions adjusted between pH 6.8 and 4.0 produced a rapid drop in viscosity due to breakage of glycosidic bonds adjacent to methoxyl groups by transelimination. Therefore, elevated temperatures near neutral pH would provide conditions very favorable for transelimination reactions and cause softening in potatoes as was suggested by Hughes et al. (1975). The softening caused by heat treatments at low pH and the heat-induced softening of carrots, a tissue containing pectins of low methoxyl ester content, would probably not be due to this transelimination effect.

It is widely recognized that freezing brings about changes in the texture of fruits and vegetables. There exists a close relationship between structure and texture that is altered by the freezing process. A summary of the structural damage damage caused in green beans due to different type of freezing can be found in an investigation by Brown (1967) where he correlated the degree of texture loss with the extent and type of damage. In this work, it was shown that although blanching and cooking did not produce visible tissue damage, freezing of the tissue did. Slightly slower than optimal freezing rates caused damage to immature cells of the inner parenchyma, as viewed by optical microscopy. Even slower rates of freezing caused breakage of other cell walls in the tissue and separation of the walls in the outer part of the pod.

Brown (1977) stated in his review of frozen fruit and vegetable structure that although blanching does not seriously alter the macrostructure of the vegetable tissue, it does allow more damage to be done by subsequent freezing. Crivelli and Buonocore (1971) reported that histological examination of blanched and unblanched tissue that had been frozen showed that blanching made the tissue more sensitive to freezing damage. They showed that the structural damage induced by freezing was more widespread in blanched than in unblanched cauliflower, green bean, carrot, and green pepper tissues.

Dietrich et al. (1959) found "no important textural changes" in green beans during frozen storage but these experiments were conducted before rapid freezing methods were in use. Brown (1977) found that textural deterioration during storage can occur due to too high storage temperature during distribution or if the material is allowed to thaw and refreeze slowly. Since cellular structure is greatly affected by freezing rate, a product thawed and refrozen in storage will be of poorer quality than one that has been maintained in a frozen state.

MATERIALS AND METHODS:

Test Materials:

Green beans (Genus species) of the Gallantin Valley 50 variety were obtained from a commercial processor. Beans were snipped and cut by the processor into approximately 3 inch lengths. The green beans were stored under refrigerated conditions ($40^{o}C$) in polyethylene bags for no more than 48 hours prior to further processing and analyses.

Processing Conditions:

Blanching - Two blanching methods were employed, steam tunnel and water bath blanching. The water bath blanch consisted of taking 100 grams of beans and placing them in 1000 ml of deionized water at approximately $100^{o}C$. After the desired blanch time the beans were removed and quickly cooled in an ice-water bath. Steam blanching was done in a steam tunnel blancher and blanch time was controlled by adjusting belt speed in order to achieve the desired residence time. The product was cooled after blanching as described above.

Freezing - Green bean samples were placed in a Conrad Blast Freezer with ambient temperature being controlled to $-69^{o} \pm 1^{o}C$. The samples were placed on wire mesh trays in a single layer in order to allow proper air circulation. Frozen samples were stored in bags and then boxed prior to being placed in frozen storage at $-20^{o}C$. The bags used for storage were heavy walled STD B620SP CRYOVAC 12" x 22" heat sealed container bags.

Thawing - Frozen samples were thawed in air for approximately 1.5 hours at $20^{o}C$ ambient temperature.

Cell Wall Preparation:

A modified method based on that described by Ahmed and Labavitch (1977) was used for preparation of cell wall material. This cell wall material, once isolated, is stable and can be stored for several months before analysis. The preparation procedure is as follows: the tissue is washed and trimmed, 100 grams is weighed and placed in a waring blender with 400 mls 70% ethanol and water and the mixture is homogenized for one minute. The slurry is transfered to several 50 ml centrifuge tubes, capped, and centrifuged at 19,000xg for 10 minutes. The supernatant is discarded and the pellet is transfered to a course sintered glass funnel. The pellet is disrupted with a metal spatula and washed three times with 100 ml volumes of 70% ethanol, three times with 100 ml volumes of chloroform:methanol(1:1), and three times with 100 ml volumes of acetone. The residue is then air dried at 20°C.

Moisture and Ash Analysis:

Moisture and ash contents of cell wall materials were determined as described by Owens et al. (1952).

Solubility Fractionation:

Water soluble fraction - 100 mg of isolated cell wall material was weighed into a 50 ml centrifuge tube and 20 mls 0.10 M TRIS buffer at pH 7.0 was added. The tube was capped and vigorously shaken at 20°C for 5 minutes. After shaking, the tube was centrifuged at 10,000 x g for 5 minutes and the supernatant collected. Additional 20 ml volumes of buffer were added and the supernatants pooled until negligible amounts of pectic material could be extracted from the pellet.

Chelator soluble fraction - To the water extracted pellet (from above) 20 mls of 0.10 M Na_2EDTA buffered to pH 7.0 with 0.10 M TRIS buffer were added. The pelleted material was suspended in the chelator solution, shaken, and centrifuged as described in the above procedure. The chelator supernatants were pooled after the pellet had been exhaustively extracted as described in the above procedure.

Hydroxide soluble fraction - To the water and chelator extracted pellet (from above) 5.0 ml of 1.0 N NaOH was added and this material was then transfered to a 100 ml volumetric flask and brought to volume with distilled water. The material was then stirred for 15 minutes and then filtered through Whatman #1 filter paper on a Buchner funnel. The filtrate was collected for analysis.

Each of the three pooled pectic fractions was held at 40°C until analyzed for uronide composition.

Uronic Acid Analysis:

Pectic material content (measured as anhydrouronic acid) was determined colcrimetrically by the method described by Blumenkrantz and Asboe-Hansen (1973). Corrections for the slightly pink chromogen formed when neutral sugar-containing materials are heated in the presence of H_2SO_4-tetraborate were made by subtracting blank values determined for tubes to which 0.5% NaOH, rather than m-hydroxydiphenyl, was added. A standard curve was constructed using 0 to 80 ug α-D-anhydrogalacturonic acid (Eastman Kodak Co.) per tube in the manner desribed above.

Textural Analysis:

For textural analyses of fresh and frozen/thawed samples, an Instron Universal Testing Machine model no. 1122 was fitted with a 50kg load cell and a scaled-down cup and plunger attachment similar to that described by Bourne and Moyer (1968). A cylindrical aluminaum cell 3.3 cm I.D., 3.8 cm O.D., 3.6 cm internal height, and 3.8 cm external height was constructed. This cup was mounted to a 10 cm x 10 cm base plate and compression/extrusion was achieved using a 3.0 cm diameter plunger.

A group of one inch pieces of green bean tissue was placed horizontally in the extrusion cell cup at a weight of 15.0 g per test compression. Plunger speed was held constant at 100 cm/min from a position 3.9 cm above the base of the cup to approximately 0.7 cm above the base as the sample was compressed and then extruded. Average extrusion force was obtained by taking the average plateau height of the extrusion curve replicates and expressing it in terms of kg force.

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RESULTS AND DISCUSSION:

Investigation into the relationship between pectic material loss into the blanching medium and textural alteration during the blanching process was conducted as follows. Green bean tissue was blanched for various lengths of time in a water bath blancher and the blanching medium retained. The texture of the green bean tissue was analyzed using Instron back extrusion and the blanching liquor was analyzed for pectic material content. The results of these analyses are found in table 1.0 and have been depicted in figures 1.0 through 1.2. Figure 1.0 shows the progressive decrease in extrusion force required to extrude green bean tissue that had been blanched for increasing lengths of time in the water bath. Pectic materials were shown to increase with increasing bath blanch time as is shown in figure 1.1. If the softening caused by heat treatment is then compared to the amount of pectic material released from the tissue, the curvilinear relationship of figure 1.2 is obtained. The steep portion of the curve may reflect that considerable changes and weakening of the structure of the middle lamella matrix and concomitant loss of intercellular adhesion result from release of small amounts of pectic substances. The results suggest that thermally-induced degradation of pectic substances results in a reduction of intercellular adhesion and possibly a weakening of cell walls in the green bean tissue as reflected by a loss in extrusion force required for back extrusion. After determination of the relationship between pectic substances released into the blanching medium and thermally-induced softening, an investigation of pectic substances in situ was conducted. A solubilization of pectic material within the green bean tissue was observed that also related to the thermally-induced softening previous characterized.

I CAN INCLUDE IN SITU RESULTS HERE

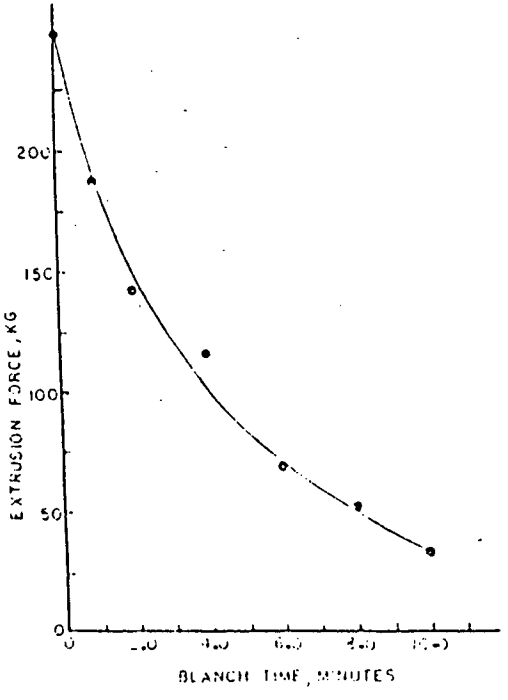
The combined textural effects of both steam blanching and blast freezing/ thawing were investigated. Tables 2.0 and 3.0 include results of textural analyses on green bean tissue that had been exposed to a variety of blanching conditions and the effects of freezing and thawing on this blanched material. The characteristic thermally-induced softening effects are evident in figures 2.0 and 3.0 where extrusion force has been plotted versus blanch time for two separate product lots. The greatest effects on product texture occur within the first 5 minutes of blanching as can be seen in figure 3.0 where blanching times were extended to 10 minutes. Blast freezing and thawing of the green bean tissue causes additional softening as measured by the back extrusion technique. This additional softening , represented by the lower curves in the two figures, is probably due to a further damaging of cell membranes and disruption of cell wall structure caused by ice crystal formation within the tissue.

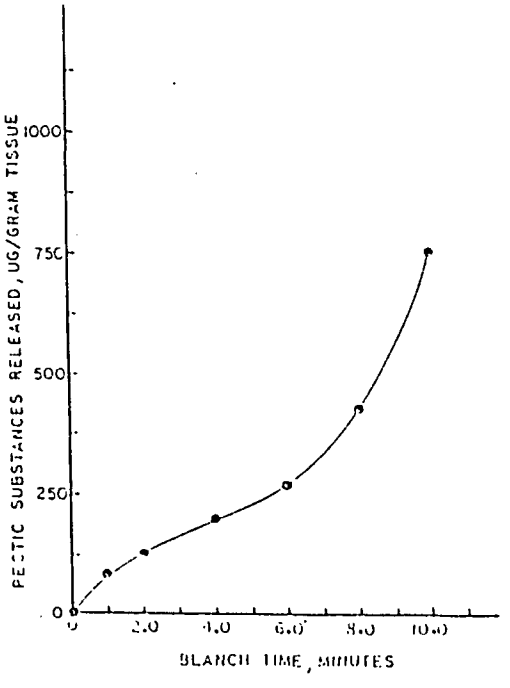
Frozen storage at -20^{o}C of blanched and unblanched green bean tissue was shown to cause no significant changes in product textural characteristics through 35 weeks of frozen storage. Table 4.0 summarizes the results of textural analyses conducted on unblanched and blanched, frozen, and thawed green bean tissues. The results of these analyses have been graphically represented in figure 4.0 and it is evident from the graph that although blanching and freezing exert a textural effect, frozen storage time does not seem to cause any noticable changes.

Analyses of the pectic polysaccharide constituents of the green bean tissues that had undergone various blanching and freezing regimes were conducted. The results in table 5.0 give the relative proportions of each class of pectic material extracted from cell wall material obtained from green bean tissues blanched for various lengths of time. No significant conclusions could be drawn from these results although the trends of increasing water soluble pectin contents in blanched fresh green bean tissue seemed to be reduced and distorted in the cell wall pectic fractionation analyses.

TABLE 1.0

FIGURES 1.0, 1.1, 1.2





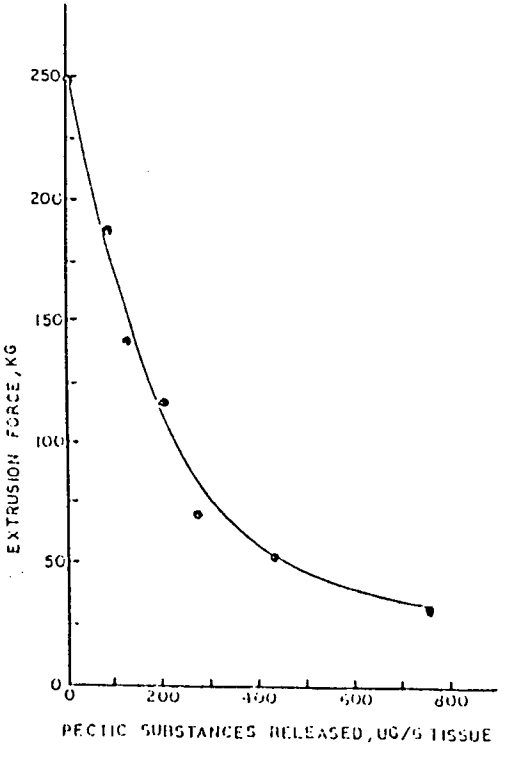


TABLE 3.0

TABLE 2.0, FIGURE 2.0

Blanch time (min)	Unfrozen green bean tissue Extrusion force (kg)	Frozen/thawed, uncooked bean tissue Extrusion force (kg)
0	202.5	131.3
1	123.0	110.0
3	74.2	56.7
5	41.3	22.5
7	30.0	17.5
10	21.7	10.8

TABLE 4.0

FIGURE 4.0

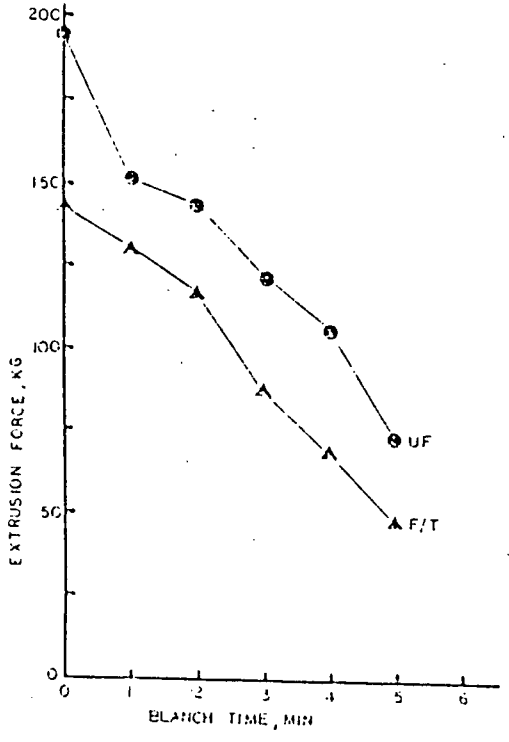
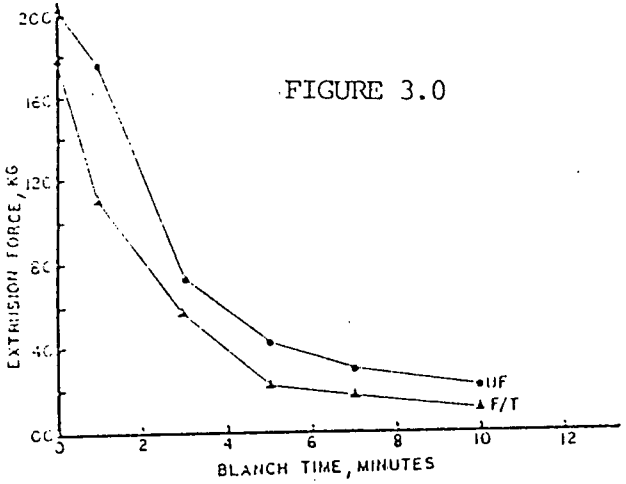


FIGURE 3.0



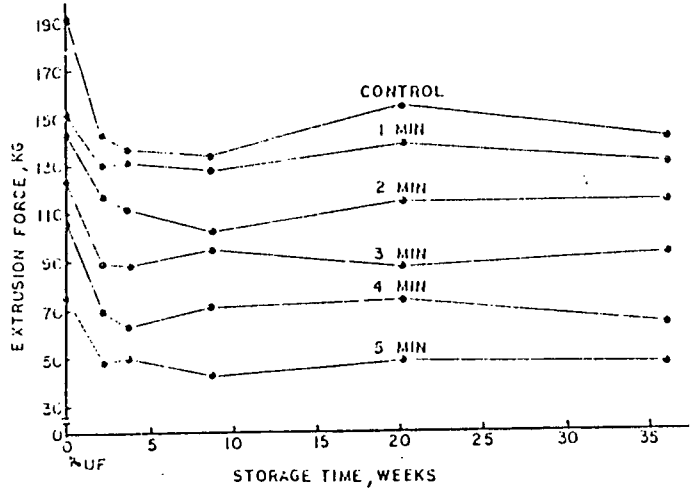


TABLE 5.0

TABLE 5. Effects of blanching and freeze-thaw on green bean cell wall composition.

[illegible] green bean tissue

Blanch time (min)	Cell wall analyses: Moisture content	Cell wall analyses: Ash content (%)	Pectic material successively extracted: Water soluble fraction	Chelator soluble fraction	Alkaline soluble fraction
0	[illegible].65	4.22	[illegible]	[illegible].83	[illegible].62
1	7.73	3.97	8.44	[illegible]	6.29
2	7.66	3.97	5.85	[illegible]	[illegible]
3	[illegible].26	4.25	7.51	[illegible]	6.15
4	6.47	3.99	9.49	[illegible].93	[illegible]
5	6.67	3.65	[illegible].15	[illegible].08	[illegible].43

Frozen thawed green bean tissue

Blanch time (min)	Cell wall analyses: Moisture content (%)	Cell wall analyses: Ash content (%)	Pectic material successively extracted: Water soluble fraction	Chelator soluble fraction	Alkaline soluble fraction
0	[illegible]	[illegible]	3.7[illegible]	[illegible]	[illegible]
1	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
2	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
3	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
4	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
5	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

TITLE:

A COMPARISON OF CALCIUM-BOUND URONIDE EXTRACTION FROM GREEN BEAN CELL WALL MATERIAL USING DIFFERENT CHELATING AGENTS.

ABSTRACT:

Several chelating agents were used to extract calcium-bound uronide material from a stable cell wall preparation. Sodium-EDTA exhibited greater extraction efficiency than the chelators, ammonium oxalate and sodium hexametaphosphate when used at 0.10 molar concentration and neutral pH. This increased efficiency for calcium-bound uronide removal at neutral pH may be due to greater solubility of the EDTA-calcium complex, stronger binding constant for Ca-EDTA, or a more effective ionization of the chelator at neutral pH. These results suggest that EDTA may be the chelating agent of choice when a non-degradative extraction of calcium-bound uronide material is desired at a neutral pH.

INTRODUCTION:

Pectic substances are part of all higher plant tissues and are particularly abundant in meristematic and parenchymous tissue(Pilnik and Voragen, 1970). They make up about one third of the dry material of the primary cell walls of fruits and vegetables and probably contribute a much greater proportion of the middle lamella(Van BUren, 1979). These pectic materials are very often associated with the textural condition of the plant tissues they are located within and are thought to be affected by ripening, as well as various fruit and vegetable processing conditions. The pectic materials are therefore often studied by researchers for their chemical and physical contributions to the textural condition of various fruit and vegetable tissue during ripening and processing treatments.

Joslyn and Deuel (1963) stated that while a considerable amount of data are available on the chemical composition of extracted pectins, investigations of the rate and extent of extraction of pectins from plant tissue preparations are limited. This, they said, is reflected in the wide variations in recommended extraction times(10 minutes to 24 hours) and temperatures(0^{o}c to boiling point of the solvent) which are found in the literature.

Because pectic substances exhibit heterogeneity, isolation is often done by fractionation into solubility groups. McColloch (1952) described detailed methods of extraction, isolation, and analysis of pectic substances based upon their solubility characteristics. He stated that the results of these solubility methods are not always reliable due to overlap of solubility characteristics. Also, no more than 70% of the total galacturonan could usually be accounted for in the fractions.

McCready and McComb (1952) have also characterized the shortcomings of such multi-extraction procedures by drawing attention to the overlapping solubilities of the various pectic substances. Thus, low methoxyl pectins may also be water soluble, depending upon the cation composition of the fruit or vegetable tissue, and on the other hand, enzyme demethylated pectins may be calcium sensitive at quite high degrees of esterification.

Robertson (1979) recently proposed a fractional extraction procedure to isolate pectic materials from alcohol insoluble solids of grapetissues and musts. In the described procedure the alcohol insoluble solids are progressively extracted by water (extracts high methoxyl pectins), ammonium oxalate(extracts low methoxyl pectins), and cold alkali(which solubilizes protopectin). Each of these three fractions can be analyzed for pectin content. This same procedure has been described by EL Tinay (1979) for the fractional extraction of guava pectic substances.

These methods provide only very conservative estimate of the proportions of each of the three fractions due to the short time of exposure and the relatively large proportion of pectic material as compared to the volume of extractant used. These methods may lack efficiency in the first two extraction steps(water and chelator extractions) and could skew the results to show a greater than accurate estimation of protopectin since material not extracted in the first two steps would be solubilized by the alkali treatment. It also seems that the final NaOH treatment could leave a certain amount of pectic material associated the the undissolved material filtered from the soluble protopectin fraction. Studies conducted in this lab have shown that acid hydrolysis of the remaining alkali treated pellet further liberates pectic material that can be quantified by uronic acid assay.

Possible reasons for the difficulties in extracting pectic substances from plant tissues have been reviewed and tested by Joslyn (1962). These include salt linkages, covalent and non-covalent bonding to hemicelluloses, and mechanical intermeshing. When pectic materials have been extracted, they often show signs of degradation due to the harsh extraction methods needed for their removal from the tissue. A lower intrinsic viscosity of these preparations is one common indicator of degradation and suggests lowered molecular weights (Van Buren, 1979).

Knee (1973) compared various degradative and non-degradative extraction methods in his research on polysaccharides and glycoproteins of apple fruit cell walls. Although the literature seems to present a variety of methods, each has its inherent advantages and disadvantages. For studies aimed at analysis of pectic materials in their non-degraded condition, extration conditions must avoid extremes in pH and concentration. Extraction of pectic materials from plant tissues exhibiting pH's in the neutral range should avoid degradative reactions associated with high or low pH conditions. This can be achieved with various buffered aqueous and aqueous chelator solutions. The choice of an aqueous chelating solution based on its ability to remove calcium-bound pectic material is discussed in this paper.

MATERIALS AND METHODS:

Test Materials:

Green beans (Genus species) of the variety Gallantin Valley 50 were obtained from a commercial processor. Beans were snipped and cut by the processor into approximately 3 inch lengths. The green beans were stored under refrigated conditions (40°C) in polyethylene bags for no more than 48 hours prior to cell wall preparation.

Cell Wall Preparation:

A modified method based on that described by Ahmed and Labavitch (1977) was used for preparation of cell wall material. This cell wall material, once isolated, is stable and can be stored for several months before analysis. The preparation procedure is as follows: the vegetable tissue is washed and trimmed, 100 grams is weighed and placed in a waring blender with 400 mls 70% ethanol and water and the mixture is homogenized for one minute. The slurry is transfered to several 50 ml centrifuge tubes, capped, and centrifuged at 19,000xg for 10 minutes. The supernatant is discarded and the pellet is transfered to a course sintered glass funnel. The pellet is disrupted with a metal spatula and washed three times with 100 ml volumes of 70% ethanol, three times with 100 ml volumes of chloroform:methanol (1:1), and three times with 100 ml volumes of acetone. The residue is then air dried at 20°C.

Extraction Procedure:

100 mg of CWM is weighed into a 50 ml centrifuge tube and 20 mls tris buffer at pH 7.0 is added before the tube is capped and shaken vigorously at 20°C for 5 minutes. After shaking the tube is centrifuged at 10,000xg for 5 minutes and the supernatant collected. Additional 20 ml volumes of buffer are added and the supernatants pooled until negligible pectic material can be extracted from the pellet.

To compare various chelating agents at neutral pH and concentration, an equal amount of identical cell wall material was successively extracted under equivalent conditions described above to remove water soluble pectic material. For each of the three chelating agents 20 mls of the chelator solution at 0.10 M and buffered with 0.10 M TRIS (adjusted with M HCl) was added to the previously extracted pellet(from above) until negligible pectin could be extracted (approximately 4 extractions with 20 mls of chelator solution). Each of the chelator solution supernatants were then held at 40°C until they could be analyzed.

Uronic Acid Analysis:

Pectic material content (measured as anhydrouronic acid) was determined colorimetrically by the method described by Blumenkrantz and Asboe-Hansen (1973). Corrections for the slightly pink chromogen formed when neutral sugar-containing materials are heated in the presence of H_2SO_4-tetraborate were made by subtracting blank values determined for tubes to which 0.5% NaOH, rather than m-hydroxydiphenyl, was added. A standard curve was constructed using 0 to 80 ug α-D-anhydrogalacturonic acid (Eastman Kodak Co.) per tube in the manner desribed above.

Anion Exchange Chromatography:

The methods of O'Beirne et al. (1981) were followed with minor modifications. Approximately 5.0 grams of DEAE-Sephadex (pharmacia, Inc.) were swollen in 100 mls of 0.10 M phosphate buffer at pH 6.90 with gentle agitation for 48 hours. A 3 cm^2 x 10 cm column was poured and a 35u Nitex sheeting was used to support th bed material.

Samples containing approximately 7.0 mg galacturonic acid residues dissolved in 12 to 14 mls of buffer containing 0.10 M phosphate (equimolar amounts of KH_2PO_4 and $NaHPO_4$), 0.001 Na_2EDTA, pH 6.90 were layered on the column. A linear concentration profile was produced in a siphon arrangement that delivered from 0.10 M phosphate to 1.0 M phosphate to the column as operating pressure was maintained at 25 to 30 cm H_20 and flow was adjusted to approximately 50 ml/hr.. Samples were collected in 5 ml tubes and held at 40°C until analyses were conducted on them.

RESULTS AND DISCUSSION:

Figure 1.0 shows relative extraction profiles for each of the chelating solutions examined. It can be noted that Na_2EDTA showed the most efficient extraction of uronide material in each of the four successive chelator extractions. The Na_2EDTA solution also demonstrated the greatest overall extraction of calcium-bound uronide material from the cell wall preparations at neutral pH.

One may postulate the reason why EDTA demonstrates this superior performance as a calcium chelator under these test conditions. If one examines the stability constants of various metal chelates (See figure 2.0) it can be seen that the interaction between EDTA and the calcium ion is very strong in comparison with various other chelator-ion complexes. This strong association may give EDTA its increased extraction efficiency above other chelating agents examined.

Another area of concern is pH's effect on ionization and in turn on chelator extraction efficiency. Since calcium extraction is an ionic charge mediated reaction, proper chelator ionization seems essential. Increased extraction efficiency by EDTA at neutral pH may be a result of a more efficient ionic condition.

One possible explanation for a lower extraction efficiency by ammonium oxalate can be based on the insolubility of an intermediate compound in the calcium exchange reaction. Calcium oxalate demonstrates a relatively low solubility and therefore a high tendency for precipitation within biological tissues(Ferguson, et al., 1980; Nancollas, 1977). It may, therefore, not affect an efficient exchange of calcium from the pectic molecules within the cell wall matrix since it may insolubilize and not carry the calcium into the extraction medium having low calcium concentration. Figure 3.0 depicts this theoretical ionic exchage relationship.

In summary, neutral extraction pH for chelator extration of calcium-bound uronide material may be desirable for avoiding dgradative reactions associated with high and low pH uronide extraction conditions. At neutral pH conditions higher extraction efficiency is demonstrated by EDTA when compared to oxalate and hexametaphosphate chelating agents at 0.10 molar concentration. The types of uronide material may be identical with each of the chelating agents and vary only in the relative amount extractable or on the other hand may vary also in the material's ionic charge distribution and molecular conformation.

Physical entrapment and slow diffusion play a major role in the freeing of uronide material from biological tissues but selection of the proper chelating agent may also be important in non-degradative extraction of the calcium-bound uronide material in the tissue.

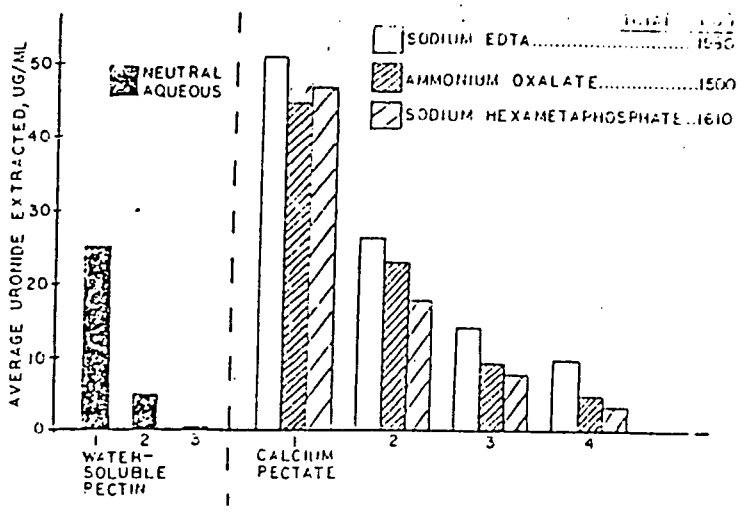


FIGURE 1.0

Extraction of pectic substances from green bean cell wall material using various chelating agents. Comparison of 0.10 M sodium EDTA, 0.10 M ammonium oxalate, and 0.10M sodium hexameta phosphate. (pH buffered to 7.0 with 0.10 M Tris-HCl)

FIGURE 2.0 LOG K_1 Stability constants of various metal chelates

(Ref. Handbook of food additives, 2nd ED. p. 275)

EDTA	Ca	10.70
Oxalic acid	Ca	3.0
Polyphoshate	Ca	3.0
Tetrametaphosphate	Ca	6.5

FIGURE 3.0 Cell wall matrix ion exchange relationship(theoretical)

HIGH CALCIUM CONCENTRATION — LOW CALCIUM CONCENTRATION

Ca-Pectate $\overset{\text{(Calcium Exchange)}}{\rightleftharpoons}$ Ca-oxalate (INSOLUBLE) $\rightleftharpoons$ Ca^{++} Oxalic acid(oxalate)

TITLE:

PHYSICOCHEMICAL CHANGES OCCURRING IN STRAWBERRY TISSUE DUE TO FREEZING AND FROZEN STORAGE.

ABSTRACT:

Frozen storage at -20°C and subsequent thawing of strawberries (Genus species) was shown to cause a decrease in the amount of total cell wall uronide in tissue cell wall preparations. Fractionation of pectic materials isolated from these cell wall preparations showed that the decrease in total cell wall uronide was mainly due to a decrease in water-soluble uronide material. Loss of pectic material in drip loss could account for the drop in total and water-soluble fractions and may be promoted by solubilization of pectic material during frozen storage in acidic tissue pH or by migratory ice recrystalization which may damage cellular architecture and allow pectic materials to become increasingly water-soluble. The texture of strawberries thawed after prolonged frozen storage at -20°C was also shown to decrease slightly by 39 weeks. This apparent change in texture may relate to the physicochemical changes occurring in the pectic cell wall materials noted above.

Anion exchange chromatography of water-soluble pectic materials isolated from the thawed strawberry cell wall preparations showed a difference between unfrozen, frozen/thawed, and frozen/thawed/stored 35 weeks at -20°C samples. A decrease in a discrete pectic fraction of greater acidic nature was observed after freezing/thawing and an apparent loss of this fraction had occurred in the long term frozen storage sample. This decrease and loss may suggest degradation of the pectic substances within strawberry tissue during freezing/thawing and frozen storage.

INTRODUCTION:

It is widely recognized that freezing brings about changes in the texture of fruits and vegetables. There exists a close relationship between structure, (localized chemical composition??), and texture that is altered by the freezing process.

The texture of both the fresh and frozen strawberry is largely determined by at least three factors that include (1) the turgidity of the cells, (2) the type and amounts of cellular contents, and (3) the structural integrity of the epidermis, parenchyma, and vascular tissue (Armbruster, 1967). Although some of these factors depend on variety, many textural changes associated with the freezing of strawberry tissue depend on the freezing treatment employed.

Brown (1966) pointed out that damage to structure and texture during freezing of fruits and vegetables is largely due to crushing or tearing of tissue during ice crystal formation. Microscopic examination of strawberries reveals that during the freezing process the parenchyma cells are ruptured but not separated while the epidermal and xylem cells are unruptured. Less severe cellular structure changes occur in berries with smaller parenchyma cells according to Armbruster(1967).

Frozen strawberries stored at or near conventional temperature of -18^{o}C are not completely frozen and still possess a certain unfrozen water content. The major physical change that occurs during frozen storage, besides sublimation, is ice recrystallization. This is a common event even at storage temperatures of -18^{o}C (Fennema, 1975). This process may be responsible for an increase in the amount of thaw exudate from a tissue as the time of frozen storage is extended, although reactions of a chemical nature may also play a role. Poor water-holding capacity in the frozen thawed product is usually accompanied by a loss of up to 80% of the original berry firmness. Rao (1967) showed that the amount of juice exuded by frozen-thawed product correlated with the soft texture of the product.

Woodroof and Shelor (1947) reported that frozen strawberry texture is better preserved at -10°F than at higher or at fluctuating temperatures. Recrystallization may cause an increase in ice crystal size during temperature fluctuation, or in other words, growth of large ice crystals at the expense of smaller ones according to Brown (1977). This effect may have caused an increase in loss of fluid after thawing as was observed by Wolford et al. (1971) in strawberries that had accidentally been exposed to warming to -7°C temperature during frozen storage.

Although frozen strawberries retain much of their original aroma, flavor, and color they suffer serious textural degradation. Work conducted by Wade (1964) showed, however, that the composition of insoluble cell wall polysaccharides was not changed by freezing. In the area of frozen storage, Wade (1964) did show a progressive decrease in the proportion of insoluble anhydrouronic acid (AUA, an estimation of insoluble cell wall pectin content) in the cell wall with increasing time of frozen storage at -18°C through 40 weeks. He attributed the loss to probable solubilization of the pectic materials in weakly acidic strawberry tissue environment. Reeve (1970) later mentioned Wade's results and commented on the loss of pectin in the cell wall as being related to a"progressive softening".

Changes in pectic substances, readily extractable components of the cell walls of fruit and vegetable tissues, have often been associated with the textural changes occurring during the ripening and commercial processing of fruit and vegetable tissues. This paper describes efforts to characterize the physical changes occurring during freezing, frozen storage, and thawing of strawberries. In addition, the cell wall material of the processed strawberry tissue was examined for changes in pectic composition and an effort was made to relate the physical and chemical changes observed.

MATERIALS AND METHODS:

Test Materials/Processing:

Strawberries (Genus species) of the Aiko variety were obtained from a commercial processor. After harvest they were mechanically topped, held for less than 24 hours at 40°C and then individually quick frozen (IQF) in a blast freezer at -69°C ± 1°C. These berries were stored in heat sealed bags to reduce sublimation in the tissue samples. Various physical and chemical tests were conducted on the samples after various lengths of storage at -20°C by removing the strawberries from frozen storage and thawing them in air at 20°C ± 1°C.

Cell Wall Preparation:

A modified method based on that described by Ahmed and Labavitch (1977) was used for preparation of cell wall material. This cell wall material, once isolated, is stable and can be stored for several months before analysis. The preparation procedure is as follows: the tissue is washed and trimmed, 100 grams is weighed and placed in a waring blender with 400 mls 70% ethanol and water and the mixture is homogenized for one minute. The slurry is transfered to several 50 ml centrifuge tubes, capped, and centrifuged at 19,000xg for 10 minutes. The supernatant is discarded and the pellet is transfered to a course sintered glass funnel. The pellet is disrupted with a metal spatula and washed three times with 100 ml volumes of 70% ethanol, three times with 100 ml volumes of chloroform:methanol (1:1), and three times with 100 ml volumes of acetone. The residue is then air dried at 20°C.

Moisture and Ash Analysis:

Moisture and ash contents of cell wall materials were determined as described by Owens et al. (1952).

Solubility Fractionation:

Water soluble fraction - 100 mg of isolated cell wall material was weighed into a 50 ml centrifuge tube and 20 mls TRIS buffer at pH 7.0 was added. The tube was capped and vigorously shaken at 20°C for 5 minutes. After shaking, the tube was centrifuged at 10,000 x g for 5 minutes and the supernatant collected. Additional 20 ml volumes of buffer were added and the supernatants pooled until negligible amounts of pectic material could be extracted from the pellet.

Chelator soluble fraction - To the water extracted pellet(from above) 20 mls of 0.10 M Na_2EDTA buffered to pH 7.0 with 0.10 M TRIS buffer were added. The pelleted material was suspended in the chelator solution, shaken, and centrifuged as described in the above procedure. The chelator supernatants were pooled after the pellet had been exhaustively extracted as described in the above procedure.

Hydroxide soluble fraction - To the water and chelator extracted pellet(from above) 5.0 ml of 1.0 N NaOH was added and this material was then transfered to a 100 ml volumetric flask and brought to volume with distilled water. The material was then stirred for 15 minutes and then filtered through Whatman #1 filter paper on a Buchner funnel. The filtrate was collected for analysis.

Each of the three pooled pectic fractions was held at 40°C until analyzed for uronide composition.

Total Cell Wall Uronide Analysis:

For determination of the total cell wall material uronide content, the method of Ahmed and Labavitch (1977) was used. This method uses a sulfuric acid hydrolysis of the CWM in order to free uronide material for analysis. The efficiency of this method in freeing uronide material from the cell wall matrix seems to be greater than that of other methods that employ enzymatic hydrolysis.

Uronic Acid Analysis:

Pectic material content (measured as anhydrouronic acid) was determined colorimetrically by the method described by Blumenkrantz and Asboe-Hansen (1973). Corrections for the slightly pink chromogen formed when neutral sugar-containing materials are heated in the presence of H_2SO_4-tetraborate were made by subtracting blank values determined for tubes to which 0.5% NaOH, rather than m-hydroxydiphenyl, was added. A standard curve was constructed using 0 to 80 ug α-D-anhydrogalacturonic acid (Eastman Kodak Co.) per tube in the manner desribed above.

Anion Exchange Chromatography:

The methods of O'Beirne et al. (1981) were followed with minor modifications. Approximately 5.0 grams of DEAE-Sephadex (pharmacia, Inc.) were swollen in 100 mls of 0.10 M phosphate buffer at pH 6.90 with gentle agitation for 48 hours. A 3 cm^2 x 10 cm column was poured and a 35u Nitex sheeting was used to support th bed material.

Samples containing approximately 7.0 mg galacturonic acid residues dissolved in 12 to 14 mls of buffer containing 0.10 M phosphate (equimolar amounts of KH_2PO_4 and $NaHPO_4$), 0.001 Na_2EDTA, pH 6.90 were layered on the column. A linear concentration profile was produced in a siphon arrangement that delivered from 0.10 M phosphate to 1.0 M phosphate to the column as operating pressure was maintained at 25 to 30 cm H_2O and flow was adjusted to approximately 50 ml/hr.. Samples were collected in 5 ml tubes and held at 40°C until analyses were conducted on them.

Textural Analysis:

For textural analyses of fresh and frozen/thawed samples, an Instron Universal Testing Machine model no. 1122 was fitted with a 50kg load cell and a scaled-down cup and plunger attachment similar to that described by Bourne and Moyer (1968). A cylindrical aluminaum cell 3.3 cm I.D., 3.8 cm O.D., 3.6 cm internal height, and 3.8 cm external height was constructed. This cup was mounted to a 10 cm x 10 cm base plate and compression/extrusion was achieved using a 3.0 cm diameter plunger. Strawberry tissue cross-sectioned to 0.5 cm width and then quartered was placed horizontally in the extrusion cell cup at a weight of 15.0 g per test compression. Plunger speed was held constant at 100 cm/min from a position 3.9 cm above the base of the cup to approximately 0.7 cm above the base as the sample was compressed and then extruded. Average extrusion force was obtained by taking the average plateau height of the extrusion curve replicates and expressing it in terms of kg force. Extreme replication was conducted in order to minimize the effects of maturity differences and textural variation within berries themselves. Berries of average maturity were chosen in each case as described by Szczesniak and Smith (1969) and then randomly split into sample groups.

RESULTS AND DISCUSSION:

In order to investigate both textural and cell wall compositional changes occurring during frozen storage of the strawberry tissue, samples were thawed at different points during the frozen storage period. In each case, the thawed sample was subdivided into two parts, one was assessed for textural properties and the other was prepared for cell wall analysis.

The results of textural analyses appear in table 1.0 and are graphically represented in figure 1.0. The graph shows a general decreasing trend in the firmness of the thawed product with increased storage time through 39 weeks. This may be due to changes in the physical structure of the product during frozen storage as ice recrystallization occurs within the tissue. Pectic materials, thought to be responsible for intercellular adhesion(cohesion??) in many plant tissues, may also undergo changes that could influence the textural properties of the thawed strawberry product.

The results of various cell wall analyses appear in table 2.0. Figure 2.0 shows a decrease in total cell wall uronide with freezing and subsequent frozen storage at -20^{o}C. This decrease for frozen/thawed samples supports previous work by Wade (1964) who stated a decrease in the amount of pectic material in the cell walls of strawberries that had been stored in the frozen state at -18^{o}C through 40 weeks.

A better understanding of what was contributing to this loss in pectinaceous cell wall material was obtained by a fractional analysis of the cell wall pectic material. The results of how strawberry tissue pectic composition in the cell wall is altered during frozen storage appear in table 3.0. These results can also be found in figure 2.0 where a comparison between total cell wall uronide and individual uronide fractions can be made. It can be seen from the figure that the greatest loss in any one pectic fraction durinf freezing/thawing and

extended frozen storage occurs in the water soluble fraction of pectic polysaccharide. This loss parallels the decrease in total cell wall uronide content and accounts for the loss in cell wall pectin for both freezing/thawing and increased frozen storage at -20°C. The other pectic fractions present in the cell wall pectic material show only slight reduction during freezing/thawing and extended periods of frozen storage as can be seen from the figure.

The increased loss of pectic material during frozen storage may be closely related to the decrease in textural properties of the thawed strawberry material. Whether or not a cause and effect relationship exists is debatable but several characteristics seem apparent. Increased drip loss of long-term frozen stored products may account for the loss of water-soluble pectic material from the strawberry cell wall material. Recrystallization of ice may be the driving force causing more extensive ice crystal damage within the frozen tissue. This possible increase in cell wall damage may cause a loss of pectinaceous material upon thawing of the strawberry tissue. Textural alterations and loss of firmness within the tissue may be solely due to a physical destruction of cell wall architecture or may be due to a release of critical pectinaceous material that had previously been responsible for intercellular adhesion(cohesion??) and structural rigidity between and within the cell wall.

One final characteristic of the system is that, even at a frozen storage temperature of -20°C, the acidic nature of the strawberry tissue provides an environment that may promote solubilization of teh pectic material in the cell wall and middle lamella. Increased frozen storage time may cause an increase in the soluble pectic polysaccharide that would be subsequently lost upon thawing into the drip loss. It would, therefore, no longer be present to confer intercellular adhesion or structural support to the molecular arrangement of the cell wall.

In order to examine more closely the changes taking place in the water soluble pectic polysaccharide fraction during freezing/thawing and extended frozen storage, anion exchange chromatography was employed. Separate pooled water-soluble pectic material fractions from unfrozen , frozen/thawed, and frozen/stored 35 weeks at -20°C/thawed strawberry cell wall preparations were chromatographed on DEAE - Sephadex A-25. Figures 3.0 throgh 5.0 show elution profiles in a linear gradient between 0.10 and 1.0 M phoshate at pH 6.90.

A large proportion of the water soluble pectic preparation initially placed on the column was eluted by the column starting buffer (0.10 M phoshate) and has not been shown. The acidic pectic material which eluted from the column between 0.1 and 1.0 M phosphate appeared to be in two major fractions eluting at different ionic strengths. This bimodal distribution is especially apparent in figure 3.0. Differences between the chromatograms are mainly found in the latter portion of the elution profiles. It can be seen that the second acidic fraction is present to a lesser extent in the frozen/thawed water-soluble sample and seems to be virtually lost in the frozen/thawed long term storage sample. The disappearance of this discrete pectic fraction may suggest a change in the normal water soluble pectic polysaccharide fraction of the strawberry cell wall material due to freezing, storage, and thawing. These characteristic changes and loss of pectic material suggested by these results may be closely associated with the textural alterations normally observed in the freezing and frozen storage of strawberry fruit tissue.

TABLE 1.0

TABLE 2.0

TABLE 3.0

FIGURE 1.0

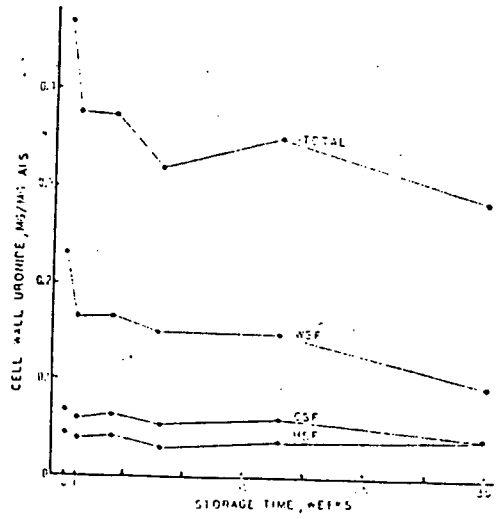


FIGURE 2.0

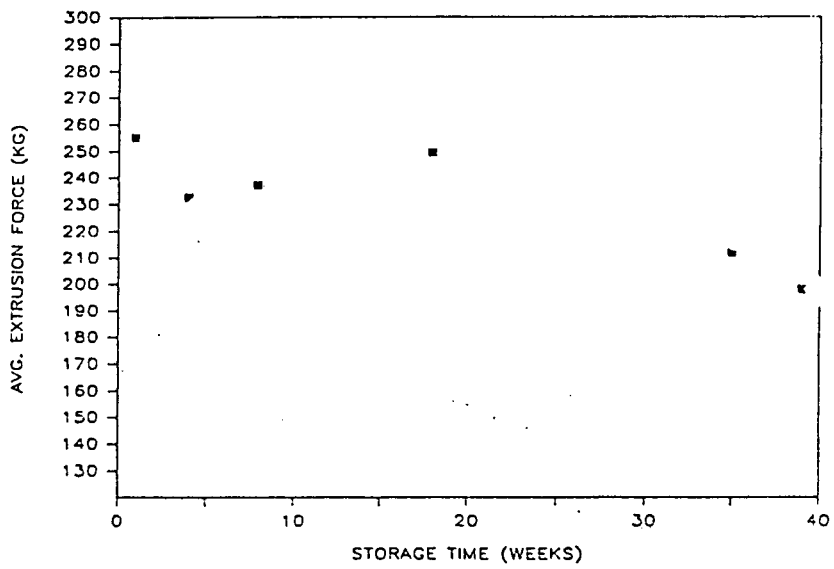


FIGURE 3.0

Fig. 33. Column chromatography of water-soluble pectin extracted from [illegible]

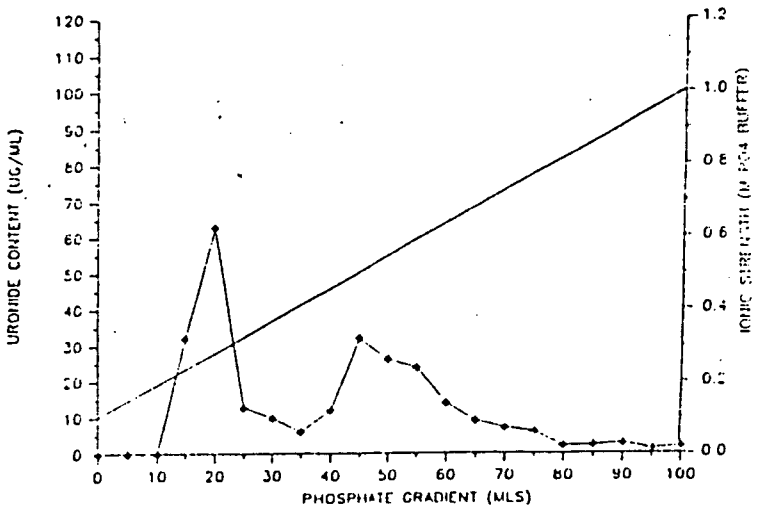


FIGURE 4.0

Fig. 34. Column chromatography of water-soluble pectin extracted from frozen/thawed strawberry tissue cell wall material. LKB-Gephadex A-25, 0.10 - 1.0 M phosphate gradient, pH 6.90

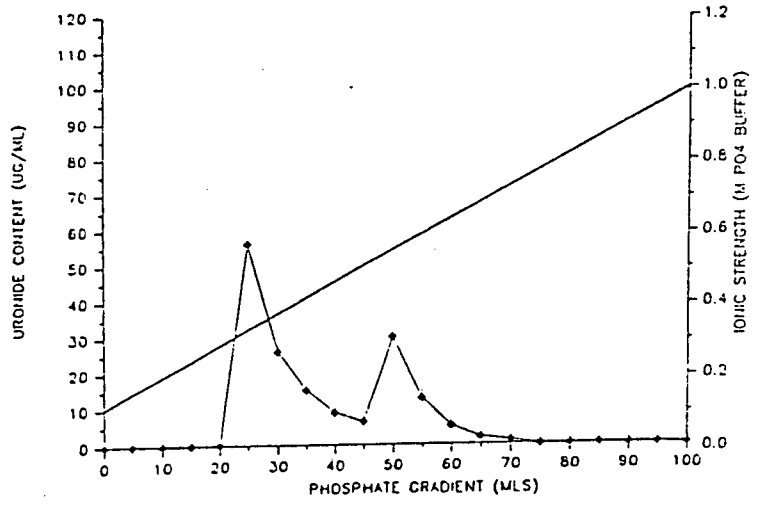
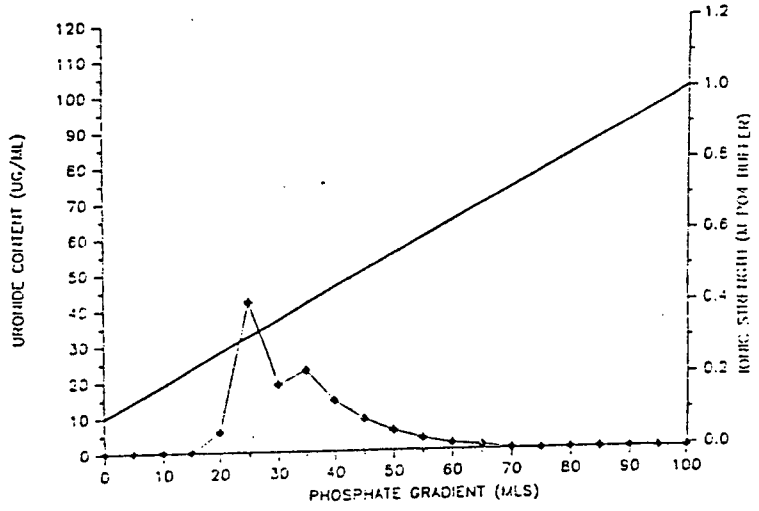


FIGURE 5.0

Fig. 35. Column chromatography of water-soluble pectin extracted from frozen/thawed,35 week storage -20°C) strawberry tissue cell wall material. LKB-Gephadex A-25, 0.10 - 1.0 M phosphate gradient, pH 6.90.



Enzyme, Texture, and Quality Changes in Diced Carrots during Blanching, Freezing, and Frozen Storage

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Abstract

Diced carrots were steam blanched for 1-5 minutes and frozen. The effect of blanching and freezing on texture, flavor and enzyme activities in diced carrots was investigated. More than 97% of the peroxidase, catalase and pectinesterase in the diced carrots were inactivated after one min steam blanching. A great decrease in pectinesterase activity during frozen storage was observed. The firmness of the carrots decreased substantially after blanching and further decreasedafter freezing and thawing. The scanning electronmicroscopic studies showed distinct changes in cell wall materials resulting from the blanching and freezing processes. The sensory analysis on texture and flavor of the frozen stored carrots was correlated with different blanching times. From these results, it is evident that 2 min blanching is necessary to maintain maximum flavor quality with the least textural loss.

Introduction

Freezing is an important means of preserving vegetables. However, the textural and chemical changes occuring during processing and storage may affect the sensory quality of the frozen products. It is generally believed that enzyme catalyzed reactions are mainly responsible for the off-flavor development in frozen vegetables. Therefore, blanching is commonly used to inactivate the enzymes causing flavor changes.

The textural quality of many frozen vegetables is inferior to that of fresh ones. It is generally agreed that texture of vegetables can be affected by the conditions of blanching, freezing, and frozen storage (Mohr, 1974; Brown, 1977). The textural changes can be caused by the alterations in cell wall structure and membrane integrity. According to Sterling (1955), cell wall seperation and loss of rigidity in the individual cell walls during processing cause the softening of plant tissues. The turgor of tissues as one aspect of the textural quality will be lost due to the damage to cell membranes.

The purpose of the present study is to investigate the effect of blanching and freezing on textural, microstructural, and enzymatic changes in carrots as well as the effects of those changes on the overall quality after frozen storage.

Methodology

Processing

Fresh carrots of Emperalta 58 cultivar were supplied by a frozen food plant in Salinas, California. They were stored at 1°C overnight, washed and diced into 1 cm cubes. Seven kg lots of diced carrots were steam blanched at 100°C for different time intervals followed by cooling in ice. The control and blanched carrot cubes were transferred to a blast freezer at -60°C. The

freezing process was accomplished in about 15 min. The frozen carrots, sealed in 1/2 kg portions in nylon bags, were stored at -23°C in a walk-in freezer.

Enzyme study

The effect of blanching and freezing on residual peroxidase, catalase and pectinesterase activity in the extract of diced carrots were determined.

Peroxidase activity was determined by the method described by Lu and Whitaker (1974). Catalase activity was determined by the method of Bergmeyer et al. (1974). Pectin esterase was assayed by monitoring carboxyl group formation (Markovic, 1977).

Texture measurement

An Instron Universal Testing Machine model No. 1122 mounted with a 500 kg load cell was employed for the textural measurements. The measurements were carried out as described by Bourne et al. (1966). A cylindrical back extrusion cell of 38 mm inner diameter with a plunger of 30 mm in diameter was used. An approximately 12 gram sample was used for each measurement. The frozen carrots were thawed at room temperature (20°C) and equilibrated to 20°C prior to measurement.

SEM study

An ISI DS-130 scanning electron microscope (SEM) was employed. All the samples were from the phloem of the carrots. Carrot samples before freezing and after frezing-thawing were fixed in 2.5% glutaraldehyde solution in 0.1 M cacodylate buffer at pH 7.0 (Davis, et al. 1976). The fixation was accomplished in two days at room temperature. The fixed samples were dehydrated, critical point dried, fractured, and coated with gold. The observations were made on the fractured surfaces. To investigate the effect of thawing on the carrot microstructures, samples were also fixed by isothermal freeze-fixation method described by Asquith and Reid (1980).

Fixing solution contained 2.5% glutaraldehyde, 0.1 M sodium cacodylate buffer (pH 7.0) and about 30% dimethylsulphoxide. The freeze-fixed samples were thawed at room temperature, dehydrated, critical point dried, and then prepared for the electron microscopic studies as the unfrozen and frozen-thawed samples.

Sensory evaluation

For the sensory analysis the frozen carrot samples after 7 months storage at -23C were thawed at room temperature and equilibrated to 25C and then served in dessert plates. Fifteen randomly selected judges were asked to indicate on a 1 to 10 scale for flavor and textural quality. Higher score means better quality. The experiments were repeated in three consecutive days. Sample numbers were randomized in each test. A three way analysis of variance procedure was carried out and the LSD range at 5% level is calculated.

Results

Enzyme activities

Results on heat inactivation of the three enzymes after steam blanching of the diced carrots are presented in Table 1. Their activities diminished as blanching time extended from 0 to 5 min. All three enzymes found in carrots were relatively unstable to heat treatment. Under the conditions used in this study they were inactivated more than 97% after one min blanching at 100C. Pectinesterase in carrots was slightly more heat resistant than peroxidase. Five min steam blanching was able to inactivate both of them.

Catalase was inactivated to an extent more than peroxidase after one min blanching. Prolonged heating, however, was not able to further eliminate the residual activity. In a separate experiment, heat resistances of peroxidase and catalase in vitro were tested and the results (not shown) indicate that

the catalase from carrot was less stable to heat treatment than peroxidase. The results are in agreement with the work done by Baardseth and Slinde (1980). The apparent residual catalase activity may be due to the existence of inorganic catalysts in the extract.

The data in Table 2 show the effect of frozen storage on enzyme retention. It is shown that after 2 months of frozen storage, pectinesterase activity in the unblanched samples declined dramatically. The activities of peroxidase and catalase decreased slightly after frozen storage.

Textural changes

Data in Table 3 show the effect of blanching, freezing, and frozen storage on the firmness of carrots as measured by extrusion force. Longer blanching time resulted in carrots with softer texture. The average extrusion force decreased to 17.4% of the original value after 5 min blanching. The same trend was maintained after samples were frozen and thawed, and after frozen storage for seven months and thawing (Table 3). Samples blanched for a longer time were softer after freezing and thawing. However, the difference became smaller after freezing and frozen storage.

The data also show a large decrease in firmness after carrots were frozen and thawed. The unblanched carrots underwent the most dramatic change in firmness, a decrease from 183 kg to 32 kg after freezing, a loss of about 68% of its original firmness. During frozen storage, the texture continued to change. The firmness of all the samples decreased by more than 50% after 7 month frozen storage.

Microstructural changes

Fig. 1 and 2 show the effect of blanching on microstructure of the carrots. At lower magnification there were no conspicous changes in cell wall structure in samples blanched for one min, and only slight change in the

sample blanched for 5 min. At higher magnification the aggregation of cytoplasms after one min blanching was observed. Sample blanched for five min appear to crinkle more than that blanched for one min.

Fig. 3 to 5 are the SEM photographs taken from samples freezing-fixed, reflecting the effect of freezing. Fig. 6 shows the SEM photographs of the thawed samples. Pictures of low magnifications show the changes in cell wall structure due to ice formation. Large intercellular spaces and broken cell walls were observed in Fig. 3. Samples blanched for five min showed more cell shrinkage and cell wall breakage than the unblanched frozen samples. At higher magnification the coagulation of cytoplasm of unblanched samples were shown in Fig. 4 and 5. The cell wall materials were damaged after the freezing process. The severity of the damage was related to blanching time. Samples without blanching showed only small pinholes at 10 kX magnification whereas small crackings can be observed in samples blanched for five min at 2 kX magnification. Samples fixed after thawing do not display much difference to those freezing-fixed at low magnifications. It was revealed at higher magnification that the severity of damage to the cell wall materials of unblanched and blanched samples tend to be more similar after thawing (Fig. 6) than that seen in Figs. 4 and 5.

Sensory evaluation

Results on sensory changes in texture and flavor due to different treatments are shown in Fig. 7 and 8. The sample treatments resulted in significantly different scores of flavor and texture at 5% level.

Samples blanched for one min received the highest score in textural quality, as illustrated in Fig. 7. There is a consistent trend showing that samples with longer blanching time received lower texture score except for the unblanched samples which were scored significantly lower than these with one

min blanching.

Fig. 8 illustrates that samples with no blanching treatment received a significantly lower score. Samples blanched for 5 min were scored significantly lower in flavor than those blanched for 2 and 3 min.

Discussion

The study on enzyme retention shows that after 2 months of frozen storage, the activities of the three enzymes, especially pectinesterase decreased. This may be related to the instability of the enzymes to freezing and thawing, or due to the tighter binding of the enzyme to the cell walls after frozen storage. Gkinis and Fennema (1978), when studying the effect of low-temperature storage on peroxidases in green beans, observed a decrease in soluble peroxidase activity after frozen storage. One possible explanation they offered is the change in esterification state of pectins. The results we obtained are consistant with their studies.

Blanching causes damage of the cell membranes by denaturing the membrane proteins and possibly by changing the organization of the lipids, leading to the loss of textural quality contributed by cell turgor. The loss of turgor was the major reason for the great drop in firmness after one min blanching. The coagulation of cytoplasms after blanching as shown in the SEM pictures indicates cell membrane damage. This is consistent with the textural measurement.

It was shown that the firmness of carrots was still decreasing over longer heating time. This may be explained by the degradation of cell walls and the leaching of cell wall conponents. The crinkling and breakage of cell walls as shown in SEM pictures may be related to solubilization of pectic materials which may alter the configuration of other macromolecules due to the change of existing hydrogen bonds (Van Buren, 1970). Tension will be

generated or released, resulting in the cell wall crinkling.

During freezing, the salt concentration around the protein molecules in cytoplasms increases. The growth of ice crystals will affect the configuration of proteins on the interfaces. Both effects may denature the proteins to form the aggregates.

During frozen storage ice crystal growth still occurs; larger crystals may grow as smaller ones may disappear. This becomes important in frozen carrots because carrots contain about 5% sugar and hence at storage temperature there may be unfrozen liquid, which facilitates the water migration. The degradation of cell wall components may also take place during frozen storage. As a result, the cell walls and cell structures can be damaged further after frozen storage.

The samples blanched before freezing showed more cell wall damage. The softening effect of blanching on cell wall may explain the more severe damage to cell walls over longer blanching time.

The observations of the microstructural changes in this study are consistant with the result on textural studies. Lower firmness value was reflected in more cracked, crinkled and damaged cell walls. This was accompanied by aggregation of cytoplasmic materials, which is an indication of membrane damage.

The results from the sensory analysis of textural quality is consistant with the textural measurement using the back extrusion test. The result that unblanched samples received significantly lower score than those blanched for one min indicates the potential benefit of heat treatment on the textural quality of frozen carrots. Since the unblanched sample firmness as measured by the extrusion test is still the highest, the lower sensory score could be due to pectinesterase activity.

It has been claimed that the off-flavor development in frozen vegetables arise from enzymatic and nonenzymatic lipid degradation and oxidation (Baardseth, 1978). The results obtained in this study indicate that off-flavor development may be related to enzymatic reactions. Fig. 8 illustrates that blanching significantly inhibited off-flavor development. Two min steam blanching at 100C seems to be enough to inactivate the off-flavor inducing enzymes. The lower flavor scores in samples blanched longer than 2 min may be due to a greater loss of volatiles during the blanching process. Thus, a 2 min blanch optimizes flavor and maintains maximum texture.

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Table 1. Enzyme retention in carrot samples blanched for different times*

Blanching time (minutes)	0	1	2	3	5
Peroxidase retention (%)	100	2.2	.13	0	0
Catalase retention (%)	100	1.5	1.6	1.3	1.0
Pectinesterase retention (%)	100	2.9	.46	.32	0

*The average of three replications.

Table 2. Enzyme retention after 2 month frozen storage and thawing

Blanching time (minutes)	0	1	2	3	5
Peroxidase retention (%)	81.6	1.0	0	0	0
Catalase retention (%)	93.5	1.2	.88	.86	.81
Pectinesterase retention (%)	4.6	.89	.15	0	0

Table 3. Effect of Blanching, Freezing and Thawing on Firmness of Carrots as Expressed in Extrusion Force (kg)

Blanching time (minutes)	0	1	2	3	5
Extrusion force (kg)	103	107	86	59	31.8
SD	10.9	5.5	5.4	8.7	2.4
Extrusion force after freezing/thawing (kg)	58.9	48.6	35.0	26.6	20.8
SD	5.1	2.5	2.7	2.2	0.84
Extrusion force (kg) after 7 month frozen storage	29.6	23.5	15.4	11.7	7.95
SD	2.65	2.73	1.31	1.00	0.70

LIST OF FIGURES

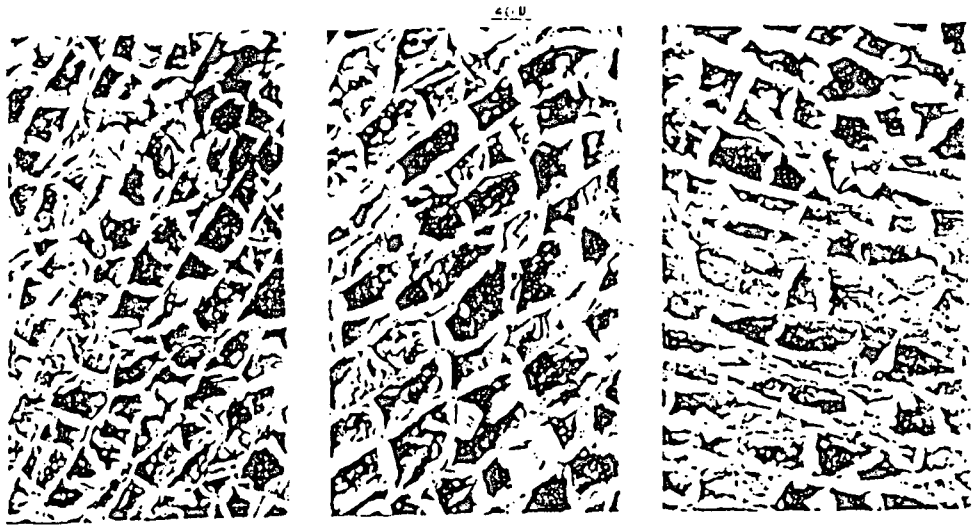


Fig. 1

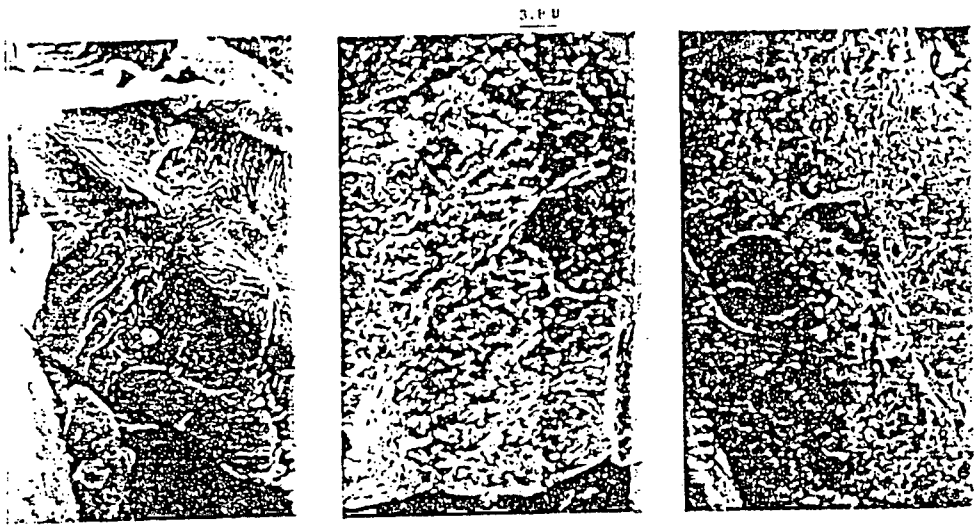


Fig. 2

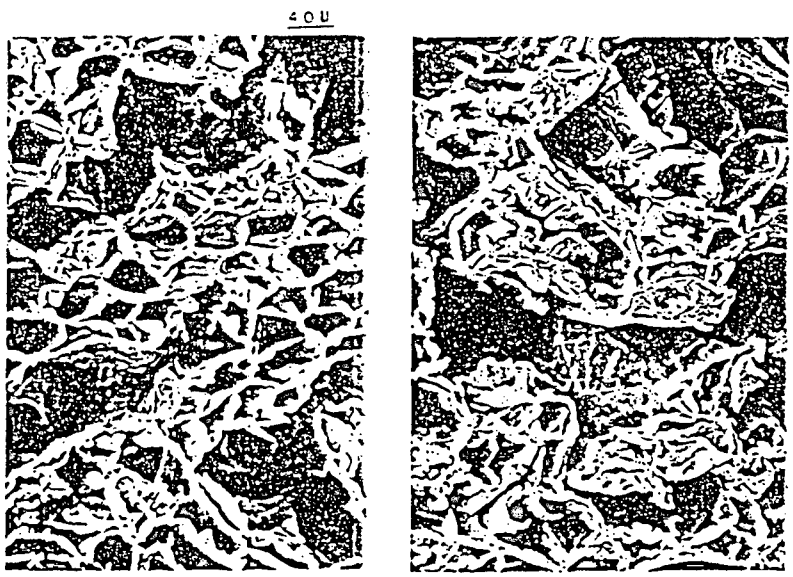


Fig. 3

Fig. 4

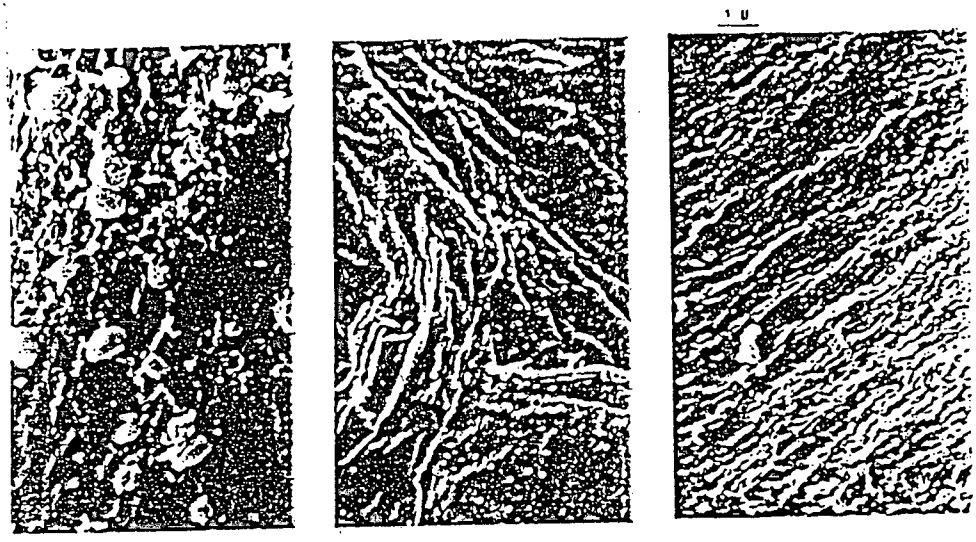


Fig. 5

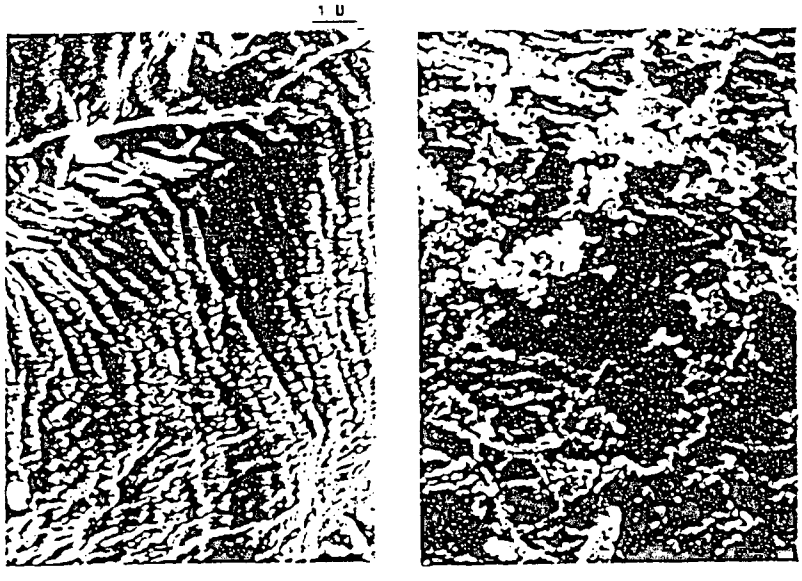


Fig. 6

Fig. 8 FLAVOR ANALYSIS

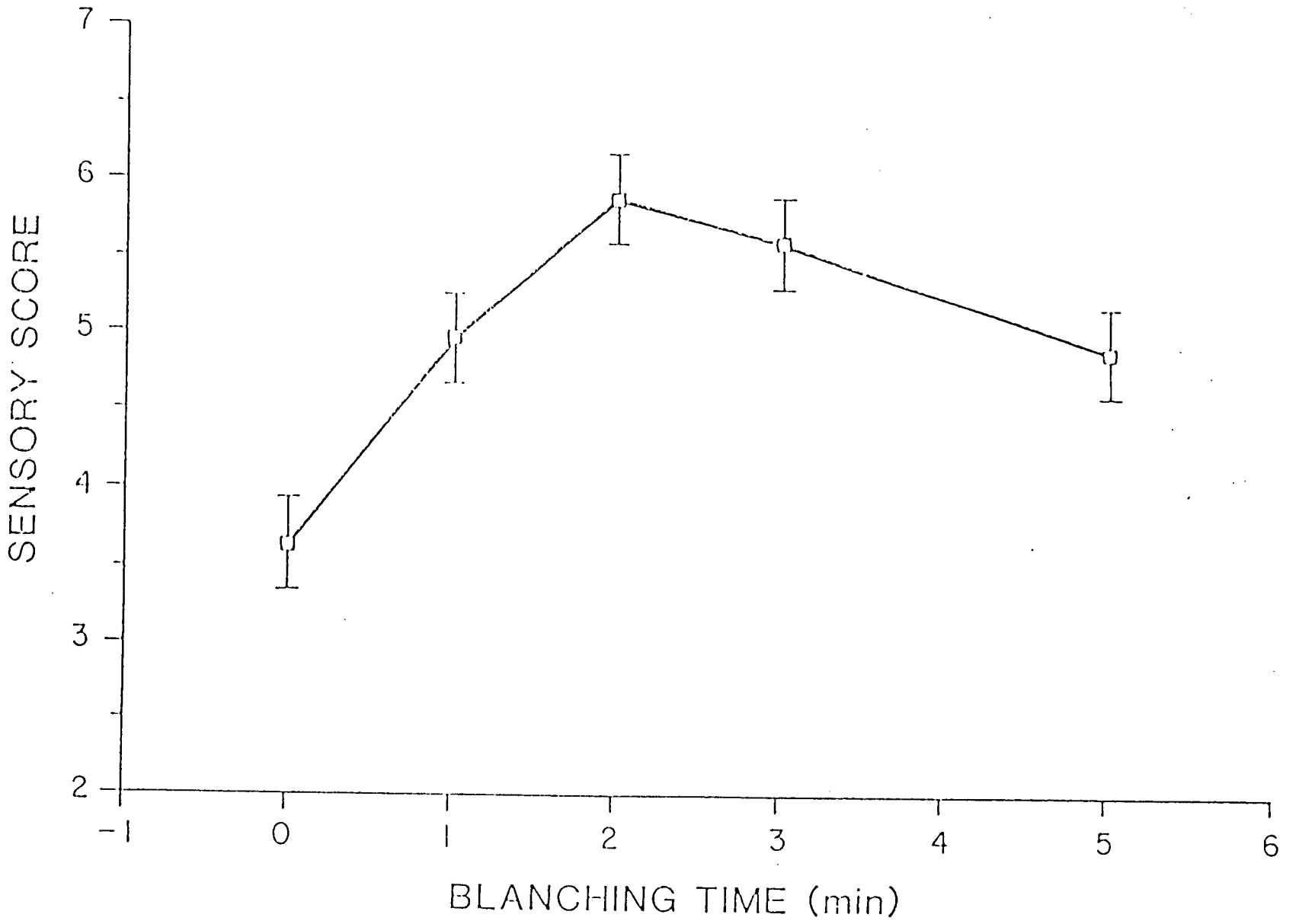
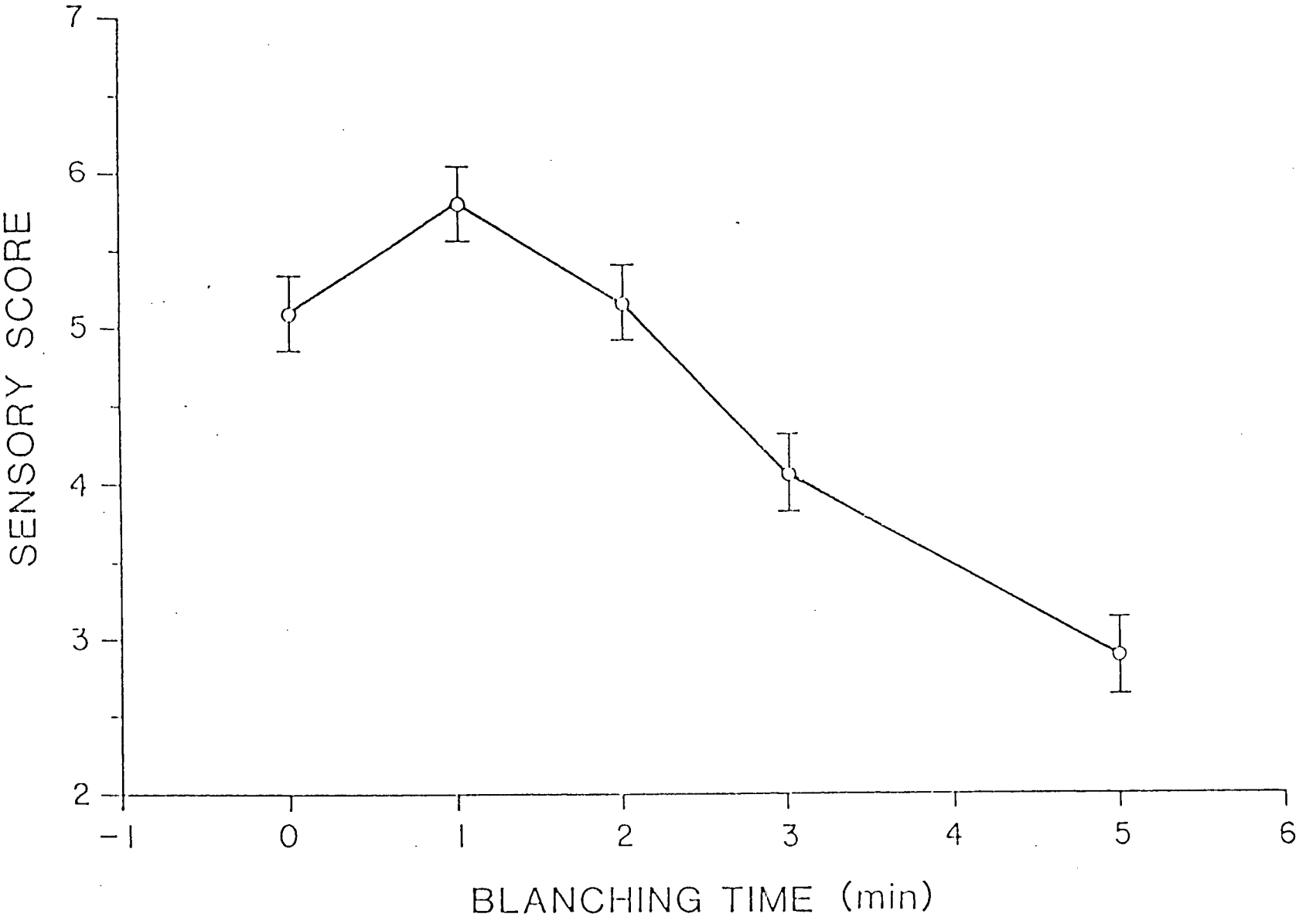


Fig.7 TEXTURE ANALYSIS



Degradation of pectic substances in carrots by heat treatment

D. Plat, N. Ben Shalom, A. Levi, D. Reid and E.E. Goldschmidt*

Abstract

Changes in the pectic substances of carrots were studied after heat treatment. Differences in soluble pectin and calcium pectate were studied after ion-exchange chromatographic separation on DEAE (diethylaminoethyl) cellulose. Different "fingerprints" were found in both pectic fractions after heat treatment. The ratio of neutral sugars to uronic acids was almost unchanged in the soluble pectin fraction, but the relative amounts of glucose and rhamnose increased after heat treatment by about ten and three fold, respectively. The ratio of neutral sugars to uronic acids in the calcium pectate increased after heat treatment from 0.11 to 0.27. On the average, all the neutral sugars increased about three fold while rhamnose increased about eight fold. The increase in the relative amount of the rhamnose compared with other sugars in the heated tissue indicates possible degradation of pectins by a B-elimination. Heating the carrot tissue resulted in degradation of the pectic substances in the cell and formation of new soluble and calcium pectate fractions. Part of these new pectic fractions derive from the original one while another part is a result of degradation.

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Introduction

PECTIN is a polysaccharide responsible for the texture of fruits and vegetables (Jarvis, 1984). The cohesion of the pectin gel is probably the critical factor in determining fruit texture (Williams and Knee, 1980, Jarvis, 1984). The structure of the cell wall polymers of the carrot root was studied intensively by Stevens and Selvendran (1984). The results of their investigation showed that the preponderant polymers in the cell wall were pectic polysaccharides with associated arabinans and galactans. The changes in the non cellulosic cell wall polysaccharides of the carrot during their growth in suspension culture was studied by Asamizu et al (1983). The polyuronoid polymers, unlike other carbohydrates, are very susceptible to degradation by B elimination upon heating at neutral or weakly acidic pH (Albersheim et al 1960; Doesberg, 1965). This reaction is catalyzed by several cations and anions (Keijbets and Pilnik, 1974; Ben-Shalom et al, 1982). Unsaturated compounds, formed by the trans elimination reaction, result from the removal of the hydrogen atom at C-5 and of the glycosidic residue at C-4 of the galacturonic acid molecule (Albersheim et al. 1960). Heat induced degradation by B elimination was found after isolation of cell wall components in potato (keijbets et al., 1976) and in cherry (Tuibault, 1983). In this study we tried to characterize the changes found in the pectic substances after heating of the carrot tissue (blanching).

Materials and Method

A ten kg batche (for each treatment) of baby carrot (var 'Amsterdam Forcing') that were obtained from the Sunfrost freezing plant in Israel was hand-peeled and divided into two samples, one of which remained untreated, while the other was steam heated (blanching) for 4 minutes, the time found necessary to inactivate the pectin esterase, PE. Alcohol-insoluble solids (AIS) were prepared from the untreated and the blanched tissue by repeated extractions with 70% and 96% alcohol. Soluble pectin was prepared by sequential extraction of the AIS with water at room temp., until no galacturonic acid appeared in the extract. Calcium pectate was extracted from the washed pellet of the asoluble pectin with 0.2% EDTA and Tris/HCl 0.02M, pH 6.2, dialyzed against water and freeze-dried.

The soluble pectin and calcium pectate (20 mg galacturonic acid) were solubilized and dialyzed with sodium phosphate buffer-1mM, pH 6.2, and applied to a column of DEAE cellulose (Whatman) 1.6X20cm which was previously equilibrated with the same buffer. Eelution was done initially with 1mM sodium phosphate (150 ml) and then with the same buffer in a linear gradient of 0-0.8M (300 ml). Fractions (3-4 ml) were collected and monitored for galacturonic acid by the m-hydroxy-phenol method (Blumenkrantz and Asboe-Hansen, 1973), and for total carbohydrate by reaction with phenol sulfuric acid (Dubois et al, 1956). Total neutral sugars were estimated from the difference between the two reactions based on galacturonic acid and glucose standards. Appropriate fractions eluted from the column were combined, dialyzed and freeze-dried.The composition and the amount of individual neutral sugars were obtained by hydrolysis in trifluoracetic acid. The respective alditol acetate were analyzed

by gas chromatography as described by Albersheim et al (1967). Methanol derived after demethylation was converted to methyl nitrite and determined by gas chromatography according to the method of Litchman and Upton (1972), as modified by Versteeg (1979).

Results and Discussion

The chromatogram of soluble pectin and calcium pectate (Fig 1,2) on the DEAE colomn showed three main fraction: The non-absorbed material (A) which was washed with 1mM phosphate buffer pH 6.2. The absorbed material which was eluted with a linear gradient of sodium phosphate (B), and the residual pectin (still bound to the column) which was eluted with 0.05M NaOH (C).

Almost all of the soluble pectin fraction of the non-treated carrot was absorbed on the DEAE column (Fig. 1_I) and eluted with the phosphate buffer with an ionic strength between 0.04M and 0.3M. Fractions A and C were negligible. Four different peaks (Fig. $1, B_{1-4}$) were separated by the phosphate gradient, they differed according to the ratio between the uronic acids and the total neutral sugars, their DE (degree of esterification) and their neutral sugars compositions.

The ratio between the galacturonic acid and the neutral sugars (Table 1) in B_1 is 0.2; in B_2, 2.5; in B_3, 15.9; and in B_4, 102.3. It can be seen from these ratios that as the ionic strength of the phosphate buffer increased the eluted pectin contained less and less neutral sugars.

Absorption of the pectic substances to the DEAE column is usually related to the DE of the polyuronide polymers (De Vries et al., 1981) and indeed peak B_4, with a DE of 47% (Fig. 1), was eluted after peak B_3, which had a DE of 67%.

Therefore, we expected that peak B_2, with DE of 100%, would be eluted before peak B_1, with DE of 44%. According to the DE of peak B_1, it was expected to elute with a ionic strength similar to that of B_4. It seems that the high amount of neutral sugars in peak B_1 in comparison with the galacturonic acid (5.2:1) has a sterical effect, preventing the interaction of functional carboxylic acid groups with the amino-groups of the DEAE column. The high ratio of neutral sugars to galacturonic acids is unusual in pectic substances, but similar results were documented in the literature by Darville et al. (1978). The absorption of B_1 on the DEAE column indicates that the neutral sugars were covalently bound to the galacturonic acid residue. Otherwise this fraction could be separated by the ionic strength of the phosphate salt.

This pectic substance rich in neutral sugars (B_1) is a good example that the absorption to the DEAE column is not dependent only on the charge of the polymer but depends also on its structure. The absorption of fraction B_2 (with 100% DE) to the DEAE column raises the possibility that this value (DE) is not due only to the methoxy groups of the carboxylic acid residues. In this case, B_2 would not have any free charged groups and would not be absorbed by the column. We assume that B_2 contained other sources of methoxy-groups, probably from phenols and neutral sugars covalently bound to the pectic polymers (Darville et al. 1978).

The soluble pectin of the heated carrot (Fig. 1_{II}) has shown a chromatogram with a completely different pattern as compared with that in the untreated carrot Peak B_1 of the untreated carrot was eluted almost at the same ionic strength as peak B of the heated carrot. Peak C of the heated carrot was greater then that of the non-heated carrot. The ratio of total neutral sugars to uronic acids in the soluble pectin was almost unchanged after heating the tissue (Table 1),

while changes were observed in the individual fraction. The ratio in peak B_1 between the uronic acid and neutral sugars was 0.2 (Table 1), compared with 2.3 in the heated one (B). The DE of peak B_1 was 44% and that of peak B (heated tissue), 30%. None of the other peaks of fraction B (B_{2-4}) of the nontreated tissue were similar to fraction B of the heated tissue in terms of ratio between uronic acid, neutal sugars and their DE values.

The composition of the neutral sugars in the peaks of fraction B of the soluble pectin (Fig. 2_I) showed that each peak is different. The difference between the peaks is clearer when comparing the sugars in each peak to the rhamnose (Table 2). The most dominant sugars in these peaks were galactose and arabinose; glucose was absent in peak B_1(Fig 2_I Table 2). The ratio between galactose and arabinose to rhamnose decreased from peak B_1 to peak B_3. These phenomena correlate with the decrease in the ratio between the neutral sugars and the galacturonic acid in these peaks (Table 1). This is due to the fact that rhamnose is mainly a part of the backbone of the polygalacturonic acid chain, and not a part of side chains of neutral sugars.

The composition of the neutral sugars of the soluble pectin in the heated tissue and their ratio are is shown in Fig. 2_{II} and Table 2, respectively. They are completely different from the control. The amount of glucose and rhamnose increased about ten and three times respectively in the heated carrot. The ratio of rhamnose to other neutral sugars increased significantly in both fractions B and C (Fig. 2_{II}, Table 2) as the relative amount of galactose and arabinose decreased. Based on these data, it seems that by applying heat to the carrot tissue (whose pH is 6.3), breakdown of part of the soluble pectin to oligomers and monomers probably occurs by a B— elimination mechanism (Albersheim et al. 1960) as shown in scheme 1. In this scheme we try to explain the effect of the

heat on the degradation and interchange of the pectic substances in the carrot tissue. The hydrolysis is catalyzed by the cell media, especially by calcium ions (Keijbets and Pilnik, 1974, Ben-Shalom et al, 1982). The oligomers formed are not found in the soluble pectin fraction of the blanched carrot, because they were extracted by the alcohol treatment. Simultaneously, part of the insoluble pectic substances of the cell wall were degraded, thus forming a new soluble pectin fraction. It is still unclear, in terms of the carrot cell wall structure and of the hydrolysis mechanism, why the new soluble fraction is rich in glucose.

Separating the calcium pectate fraction of the non-treated carrot on DEAE cellulose (Fig. 3_I) showed a chromatogram in which about 95% of the calcium pectate is in fraction B. This fraction was eluted in a broad peak with the phosphate gradient buffer between 0.07 and 0.32M. The calcium pectate fraction is characterized by a small amount of neutral sugars (uronic acids to neutral sugar ratio 9.3:1, Table 3) and low DE in the fraction (23%, Fig. 3). Fractions A and B have similar ratios of their neutral sugars to rhamnose (Table 4), but the main difference between them is the ratio of uronic acid to neural sugars which is 1.1 in A and 14.8 in B (Table 3). The relatively high sugar composition of A may be the reason that this fraction was not absorbed onto the column eventhough it has a low DE (Fig 3). From the chromatogram of the calcium pectate fraction on the DEAE and its sugar analysis, it is obvious that it is a separate fraction completely different from the soluble pectin.

Heating the carrot resulted in the formation of a different calcium pectate fraction (Fig. 3_{II}). A significant increase in the amount of neutral sugars appeared in the heated fraction. The ratio of uronic acids to neutral sugars changed from 9.3:1 (in the untreated tissue) to 3.6:1 (in the heated tissue,

Table 3). The main changes were found in fraction B, in which the neutral sugars increased by about 2.5 fold. In the neutral sugars, the rhamnose ratio increased most significantly (eight times), while the other sugars increased approximately three fold (Fig. 3_{II}, Table 4). The increasing amount of rhamnose after heat treatment, as compared with other sugars in the calcium pectate as well as in the soluble pectin, lends support to the theory that the degradation of the carrot tissue is via the B-elimination mechanism. The increase in the neutral sugars in fraction A was accompanied by a large increase in the level of uronic acid. Again the ratio to rhamnose increased most significantly, followed by that of galactose, large change was observed in the arabinose. The heat treatment did not have a big effect on the DE of the calcium pectate. The DE in fraction A was 19% and in fraction B, 15.5% (Fig. 3_{II}). The low DE in the calcium pectate of the heated carrot emphasizes the fact that calcium interacts mainly with low esterified pectic substances. Eluting the calcium pectate with NaOH (fraction C) resulted in a fraction that had a minor amount of neutral sugars, and, only a little more uronic acid (as compared with the non-heated tissue). It seems that after applying heat to the carrot tissue, new calcium pectate fraction forms (scheme 1). Part of this fraction derives from the original calcium pectate, while the rest is probably a degradation product of other, pectic substances (soluble pectin and proto pectin) which interact with calcium ions to form new calcium pectate fractions. The free calcium ions are probably derived from the cell vacuoles and membranes. They catalyze simultaneously the cell wall breakdown by B elimination reaction (Keijbets and Pilnik, 1974) and interact with part of the soluble pectic substances which have low esterified groups.

Conclusion

The changes in the pectic substances with heating seem to be a complicated process. This process is a dynamic system with various steps of degradation and the formation of several pectic fractions occurring simultaneously. The high amount of rhamnose residues as compared to other neutral sugars) in the pectic fractions which formed after heat treatment of the slighly acid pH of the carrot tissue, show that the mechanism of degradation by heating in this tissue is mainly through B elimination. The B elimination degradation has been shown in other tissues in vivo and in vitro (Doesberg 1965, Keijbets 1976, Tuibault 1983). An approach in this paper was to try to follow part of the interchanges in the pectic substances within the carrot tissue which occured through B elimination. The degradation process is probably influenced by the cell media, and by the primary and secondary structure of the pectic polysaccharides including also the spsecific bonds between molecules, such as the calcium bridge and the hydrogen bonds. More research on the structure of the pectic ploysaccharides is needed in order to determine which regions and side chains of the polymers are more susceptible to chemical changes induced by the heat treatment.

Contribution from the Agricultural Research Organization, The Volcani Center, Bet Dagan, Israel. No. 1689-E, 1986 series. The work was supported by the United States-Israel Binational Agricultural Research and Development Fund (BARD), Project No. US-452-81.

Fig. 1 Separation of soluble pectin on DEAE cellulose. The chromatogram of untreated carrot tissue (I) and of heated tissue (II), were obtained by following the content of uronic acids (●——●) and of total sugars (□——□).

Three main fractions were shown in each chromatogram: A; non-absorbed material which was washed with 1mM phosphate buffer pH 6.2 B; The absorbed material which was eluted with a linear gradient of sodium phosphate. C; the residual pection (still bound to the column) which was eluted with 0.05M NaOH. The percent which is given in the figure is the degree of esterification.

Fig. 2 Composition of neutral sugars in the soluble pection fraction of untreated carrot tissue (I) and of heated tissue (II), after their separation of DEAE cellulose column.

Fig. 3 Separataon of calcium pectate on DEAE cellulose. The chromatograms of untreated carrot tissue (I) and of heated tissue (II) were obtained by following the content of uronic acids (●——●) and of neutral sugars (□——□).

Three main fractions were shown in each chromatogram: A; none absorbed material which was washed with 1mM phosphate buffer pH 6.2 B; The absorbed material which was eluted with a linear gradient of sodium phosphate. C; the residual pection (still bound to the column) which was eluted with 0.05M NaOH. The percent which is given in the figure is the degree of esterification.

Fig. 4 Composition of neutral sugars in the calcium pectate fraction of unheated carrot tissue (I) and of heated tissue (II), after their separation on DEAE cellulose column.

Scheme 1: Changes of pectic substances in carrot tissue caused by heat treatment.

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Table 1: The content of total galacturonic acids and neutral sugars after separation on DEAE column in the soluble pectin fraction of untreated and heated carrot tissue.
The sugar content is expressed in microgram and as a percent of total pectic substances.

Treatment	Sugar Content (ug) Fraction No.												Total sugars	
	A	%	B_1	%	B_2	%	B_3	%	B_4	%	C	%	ug	%
Untreated tissue														
Neutral sugars	170	-	8929	28.7	403	1.3	531	1.7	95	0.3	35	0.1	10173	32.7
Galacturonic acids	108	0.3	1701	5.5	1008	3.2	8435	27.1	9549	30.7	250	0.8	20943	67.3
Pectic substances	108	0.3	10630	34.2	1411	4.5	8966	28.8	9647	31.0	285	0.9	31116	100.0
Blanched tissue														
Neutral sugars	294	-	7980	25.3							3250	10.3	11230	35.6
Galacturonic acids	-	-	18360	58.2							1980	6.3	20340	64.4
Pectic substances	-	-	26340	83.5							5230	16.6	31570	100.0

Table 2 The ratio between neutral sugars and rhamnose in the soluble pectin fraction of untreated and heated carrot tissue.

Neutral sugars Composition	Untreated tissue			Fraction	Heated tissue	
	B_1	B_2	B_3		B	C
Arabinose	10.0	6.9	5.0		2.9	1.9
Mannose	0.5	1.7	1.0		1.2	0.9
Galactose	16.4	9.2	6.0		4.7	3.2
Glucose	-	3.1	1.5		1.3	6.7

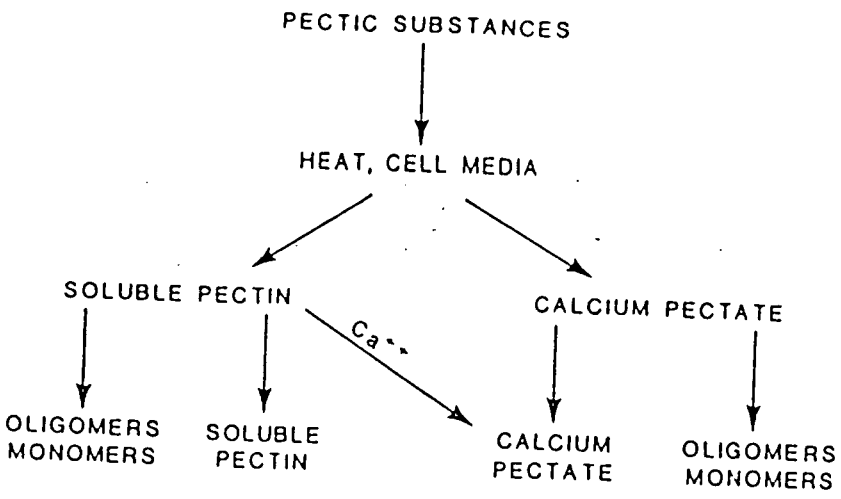
Table 3: The content of total galacturonic acids and neutral heated sugars after separation on DEAE column in the calcium pectate fracture of untreated and heated carrot tissue.

The sugar content is expressed in micrograms and as a percent of total pectic substances.

Treatment	Sugar Content (ug) Fraction No. A	%	B	%	C	%	Total Sugars ug	%
Untreated tissue								
Neutral sugars	680	3.1	1260	5.8	180	0.8	2120	9.7
Galacturonic acids	761	3.5	18720	85.6	262	1.2	19743	90.3
Pectic substances	1441	6.6	19980	91.4	442	2.0	21863	100.0
Blanched tissue								
Neutral sugars	1796	6.8	3915	14.8	24	0.1	5735	21.7
Galacturonic acids	5184	19.6	14616	55.2	954	3.6	20754	78.3
Pectic substances	6980	26.4	18531	70.0	978	3.7	26489	100.0

Table 4 The ratio between neutral sugars and rhamnose in the calcium pectate fraction of untreated and heated carrot tissue.

Neutral sugars composition	Untreated tissue Fraction			Heated tissue		
	A	B	C	A	B	C
Arabinose	5.5	5.5		0.4	1.9	
Mannose	3.0	3.0	traces	-	0.7	traces
Galactose	4.0	5.5		2.1	2.8	
Glucose	3.5	3.0		1.0	1.2	


PECTIC SUBSTANCES
HEAT, CELL MEDIA
SOLUBLE PECTIN
CALCIUM PECTATE
Ca++
OLIGOMERS
MONOMERS
SOLUBLE
PECTIN
CALCIUM
PECTATE
OLIGOMERS
MONOMERS

Scheme - 1

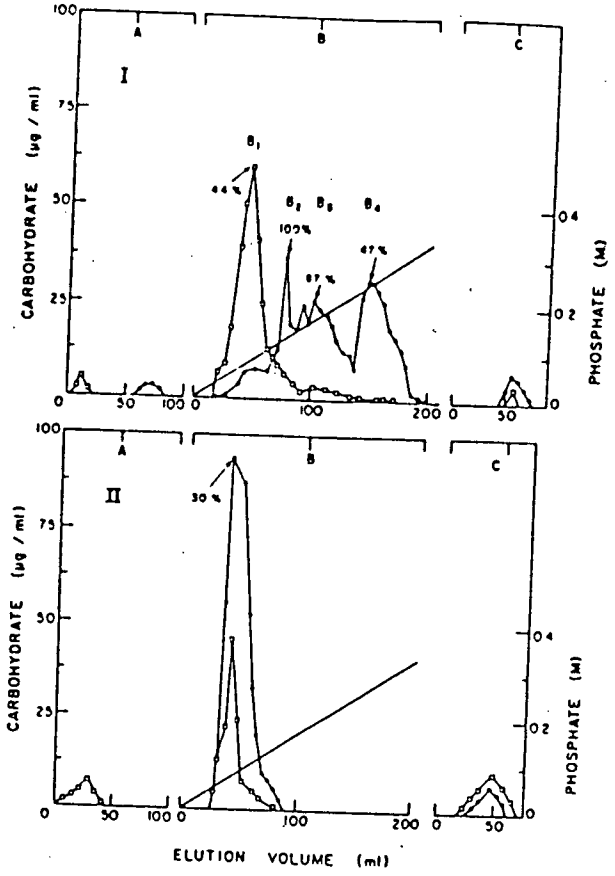

I
II
A
B
C
CARBOHYDRATE (µg / ml)
PHOSPHATE (M)
ELUTION VOLUME (ml)
44 %
109 %
97 %
47 %
30 %

Fig 1

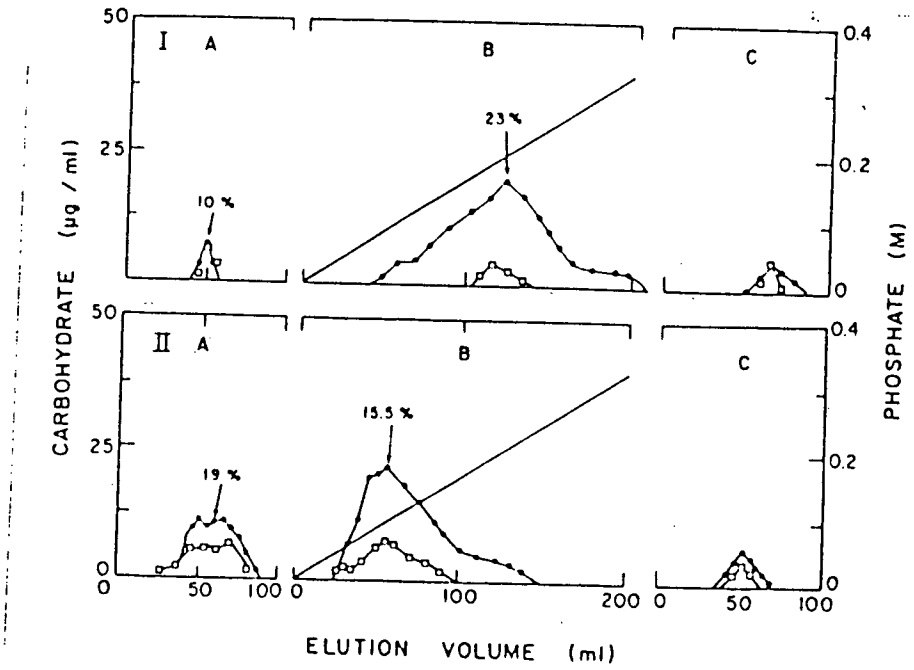

I A
B
C
II A
B
C
CARBOHYDRATE (µg / ml)
PHOSPHATE (M)
ELUTION VOLUME (ml)
10 %
23 %
19 %
15.5 %

Fig-3

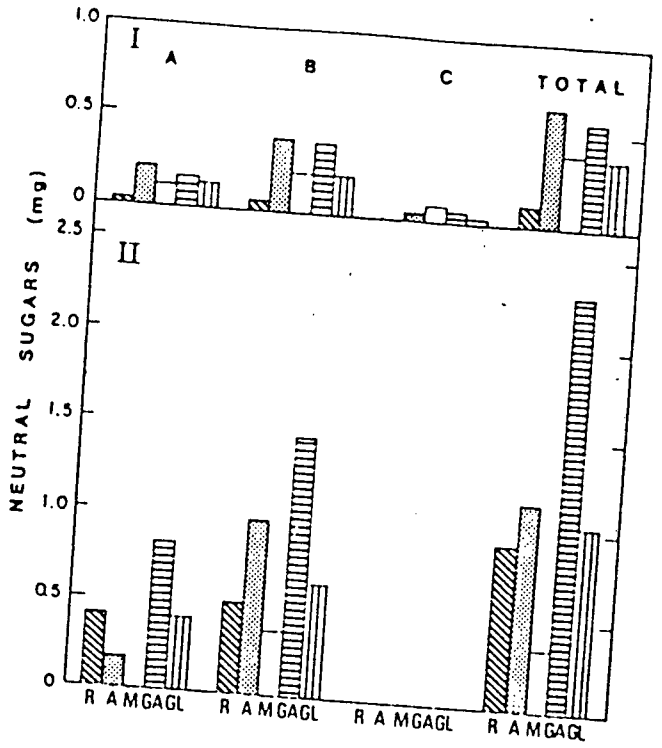

I
A
B
C
TOTAL
II
NEUTRAL SUGARS (mg)
1.0
0.5
0
2.5
2.0
1.5
1.0
0.5
0
R A M G A G L
R A M G A G L
R A M G A G L
R A M G A G L

Fig 2

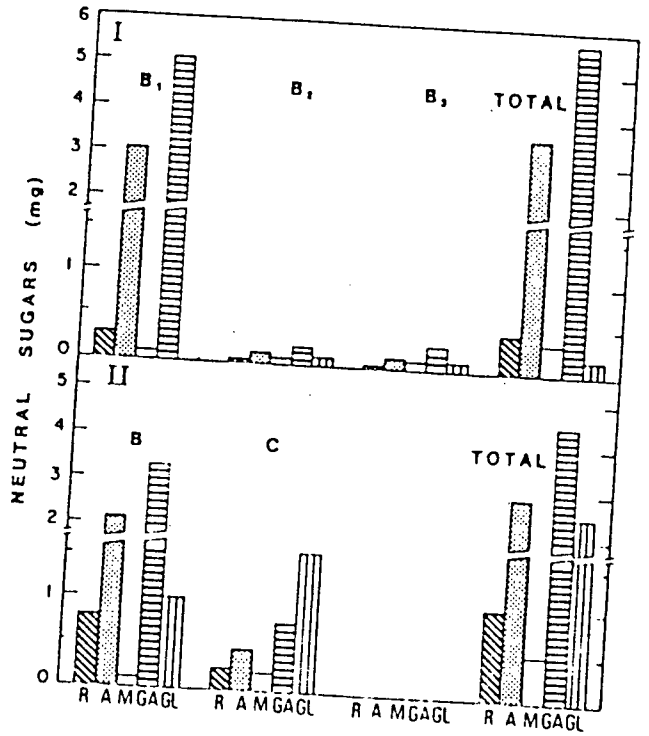

I
B1
B2
B3
TOTAL
II
B
C
TOTAL
NEUTRAL SUGARS (mg)
6
5
4
3
2
1
0
R A M G A G L
R A M G A G L
R A M G A G L
R A M G A G L

Fig 4

Effect of blanching and drying on pectic constituents and related characteristics of dehydrated peaches

Aharon Levi, Noach Ben-Shalom, David Plat and David S. Reid.

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Running head - Pectin and dehydration of peaches

Effect of blanching and drying on pectic constituents and related characteristics of dehydrated peaches.

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Abstract

The effects of sulphuring, blanching, dehydration and storage (of dehydrated fruits), on the pectic constituents and other characteristics of peaches are reported. Adequate blanching stabilized pectins of the dehydrated peaches so higher rehydration capacity and lower rehydration losses were observed after 5 min blanching. Increase in degradation was observed in non-blanched or 15 min heat-treated fruits, which resulted in lower rehydration capacity. A significant correlation was found between the contents of total pectin and protopectin fractions and the firmness or rehydration ratio of the peaches. Thus, pectin - one of the major cell-wall and intercellular tissue components, - plays a significant role in determining the textural-structural characteristics of dehydrated fruits.

Pectins and dehydration of peaches

Introduction

When plant tissue is heat treated during an industrial process, (blanching, cooking, dehydration, etc.), some physico-chemical changes of the structural constituents of the cell wall (CW) and the inter-cellular (IC) tissue are observed. The resulting textural and other behavioral characteristics of the processed tissue, would be different from those of the untreated plant tissue. Matthee and Appledorf (1978) reported that cooking liberates cellulose and pectic substances from the CW, or can even cause their hydrolysis with consequent changes in their water-binding or holding capacity.

Pectins, hemicellulose and cellulose are among the main water binding components of the plant CW in particular and the plant tissue in general. The degradation and molecular configuration of the pectins could cause structural-textural breakdown, resulting in variations in firmness, water liberation or binding, tissue disintegration, etc. Pectins are also the most susceptible to enzymatic and heat-induced degradation and configuration. Natural differences or induced changes in their physico-chemical characteristics would affect the plant CW and IC tissues, including their water-holding capacity (Parrott and Thrall, 1978).

Luh et al.,(1978) reported changes in firmness of canned apricots during storage, due to pectin degrdation, catalyzed by added poly-galacturonase (PG) of mold origin, Levi et al.,(1980) reported variation in water removal, with increasing blanching time, during dehydration of sulphited bananas. Heat treatments also affect the permeability of the cellular membranes and the CW (Rotstein and Cornish, 1978), parallely with the water binding capacity of plant

fibrous materials (Parrot and Thrall, 1978). Levi et al.(1983) found that the dehydration velocity of papaya was affected by blanching and by the dehydration temperature. Mtak and Perry (1948) reported that blanched peaches take less time to dehydrate. Differences in the dehydration capacity and other textural-structural properties, would be expected in dehydrated fruits, due to enzymatic and heat-induced changes of their pectic constitutents.

The amount of the pectic substances in fresh peaches was reported to be around 0,45% (Vidal-Valverde et al. 1982). The soluble pectin fraction in the peach tissue increased dramatically during ripening (Pressey et al., 1971). PG activity was not found in unripe peaches, but it developed during ripening, parallely to the formation of soluble pectins, the molecular weight of which decreased progressively during the ripening (Pressey and Avants, 1978). According to Shewfelt (1965), pectin esterase (PE) is present in the peach fruits at all stages of ripening, therefore, PE activity does not appear to be a limiting factor for pectin solubilization, during ripening. Postmayr et al., (1965), studied the effects of heat processing (canning for 18 min or more), on changes in some pectic fractions of peaches. They found that processing affects both the texture and the pectin content of the canned peaches.

The objective of this study was to assess the effects of sulphuring, heat pretreatments, dehydration and storage on the contents of the pectins' major fractions, and the rehydration capacity, the rehydration losses of dehydrated peaches and the firmness of blanched peaches.

Pectin and dehydration of peaches

Materials and Methods

Pretreatments and dehydration

Standard uniform sulphuring, blanching and dehydration procedures were applied in order to be able to assess their effect on the pectins, parallely with textural-structural characteristics of the so treated and dehydrated peaches. About 5 kg (for each experimental condition) of firm-ripe freestone peaches cv. 'Sommerset', were cut in half and their stones removed manually. The peach halves were subjected to heat pretreatments (blanching) in a steam blancher at atmospheric pressure for 0 to 15 min, and then immediately cooled to 30°C with gentle tape water sprays. Then the peach halves were sulphured in a sulphuring (SO_2 fumes) cabinet for 2½ h. The dehydration was done in a cabinet-dryer, at constant flow-through air velocity, on perforated aluminum trays for 3 h, at 70±5°C, followed by 7-10 h at 50±5°C, to > 82% dry matter (DM).

One part was analyzed within 2 weeks of preparation and the rest was stored for one year at ambient temperature.

Rehydration and cooking

The rehydration capacity of the dehydrated peaches was assessed as following: 50 g dehydrated peach halves was cut in small (1 cm^3) pieces, and submerged in 500 ml distilled water for 24 h at room temperature. The residual water was removed, the adhering water absorbed carefully with tissue paper, and the peaches weighed. The rehydration weight was expressed as the percentage of the initial weight per DM content of the dehydrated peaches (expressed as 100%). Data are expressed as the arithmetic average values of three separate replications for each experimental condition.

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To assess the rehydration effect on cooking, the peach pieces (as above) were submerged for 30 min in boiling water and treated as above.

The DM losses during the rehydration, were calculated by the following equation:

$$DML(\%) = \frac{(WoDMo - W1\ DM1) \times 100}{WoDMo}$$

Wo - Weight of dehydrated sample; W1 - weight of rehydrated sample;

DMo - DM content of dehydrated sample; DM_1 - DM content of rehydrated sample;

DML - Dry matter loss during rehydration.

Firmness

Five cubes of 1x1x1 cm each were compressed with an Instron model TM 1026 insturment to 50% of their height, at 5 cm/min-velocity. The instrument was calibrated with a full scale load of 50 kg. The power applied to compress the peach cubes was calculated at the peak of the compression curve in Neuton-force units. Data are expressed as the arithmetic average of three separate compressions for each experimental condition.

Pectin fractionation and analysis

Alcohol insoluble solids (AIS) were prepared within 1 h of the fresh, or treated non-dehydrated peaches, and within 1-2 weeks or one year of the dehydrated ones. The AIS were extracted by subjecting 250-300 g of the nondried or 50 g of the dried peaches to homogenization (Waring Blendor), four times in succession, with 300 ml of 70% ethanol each time, followed by a fifth extraction with 100 ml of 100% acetone at room temperature.

Pectin and dehydration of peaches

The soluble pectin (SP) was extracted at room temperature from the AIS as follows: 200 mg AIS was vigorously stirred for 10 min with 20 ml distilled water and then centrifuged at 15.000 rpm for 20 min. This procedure was repeated three to seven times with the pellet until no galacturonic acid (GA) was detected in the supernatant. The supernatants were collected and analyzed for GA content as described below and the pellet was subjected to calcium pectate (CaP) extraction, as follows: 25 ml of a solution containing 0.1M buffer TRIS/HCl and 0.2% EDTA at pH 6.2 were added to the above pellet, stirred, and centrifuged as above several times (4-7) until no GA was detected in the last supernatant. The supernatants were collected for CaP determination. The final pellet was extracted as above - for protopectin (PP), with 0.05N Na OH (pellet: NaOH = 1:50) and the supernatant was analyzed as described below. The amount of each pectic fraction was assessed colorimetrically as described by Blumenkrantz and Asboe-Hansen (1973) and calculated as GA on the AIS contents - calculated on 100% DM, or fresh tissue basis. The pectin fractions are expressed as a percentage of the total pectin (TP) content of the fresh (untreated) peach halves; which is the total added value of the PP + CaP + SP fractions.

The PE activity was determined by the method given by Levi et al., (1980).

No PE activity was detected after blanching of the peaches for 5 min or longer.

Pectin and dehydration of peaches

Results

Pectins

Water and alcohol soluble solids (ASS), such as sugars, acids, GA monomers and oligomers, etc., are removed from the tissue during the blanching-cooking and sulphuring (by dripping) process steps. The ratio of the AIS to the DM, changes accordingly (Table 1). A decrease in the DM content (also caused by major water penetration throughout the cell wall - during the cooling) - with an increase in the AIS (on DM basis) was indeed observed in the blanched-sulphured peaches in all the experiments. The results of one of the experiments are given in Table 1. Similar tendency, but of different magnitudes, was observed in all the experiments. Because of the above phenomena, it seems more accurate to compare the pectin changes on a DM basis.

The arithmetic average (two replicates) pectin contents of the peaches after the different pre-treatments dehydration and storage are given in Fig 1.

The total pectin content (TP) (Fig 1A) of fresh peaches was 2997 mg/100g DM and is expressed in Fig 1 as 100%, which is also the base for the contents given in Figures 1B, C, D.

After sulphuring only (0 min, I) a decrease of about 10% of the TP was observed - probably caused mainly by pectolytic enzymes' degradation. Dehydration of I (0 min, II), caused an additional degradation and a further significant decrease in the TP. Only 60% of the original TP remained after 1 year storage (0 min, III).

Pectin and dehydration of peaches

Blanching for 5 min (5 min, I) inactivated the pectolytic enzymes, therefore no significant decrease was observed after this pretreatment and sulphuring. Some decrease, probably due to heat-incuced degradation, was observed after dehydration of I (5 min II), while the small increase in TP observed after storage of II (5 min, III) could be a result of natural differences of the pectin content of the fresh peaches. Regardless, the short (adequate) blanching probably stabilized the pectin by inactivating the pectolytic enzymes, without causing any significant heat-induced degradation, because of the short heating time.

Heat degradation after a 15 min blanch (I), is probably the main reason for the observed decrease in the TP. The dehydration of I(II) did not cause a further decrease in the TP content, while a small decrease was observed after storage (15 min, III).

Water soluble pectin (SP) - Fig 1 B - was the major pectic fraction found in the peaches: 60% of the TP of the fresh fruit (0 min, 0). Sulphuring only (0 min, I), caused an increase of about 10% in the SP. Dehydration of the sulphured peaches (0 min, II) reduced the SP content and a further decrease was observed after 1 year's storage of the dehydrated peaches (0 min, III). Pectolytic enzymes are probalby responsible for the observed phenomena (see Discussion).

Short (5 min) heat treatment stabilized the SP and its contents remained almost the same as in fresh peaches (5 min, I). A significant decrease of the SP was observed after dehydration of I (5 min, II) probably by the heat effect, while the observed increase in SP after storage (5 min, III) could be a result of the degradation of other pectin fractions (see Discussion).

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The heat degradation effect was pronounced in the peaches heat treated for a longer time (15 min, I). further degradation was observed after the dehydration (15 min, II), but no decrease in SP was observed after storage (15 min, III).

CaP - Fig 1C was found to be a minor fraction of the fresh peaches: about 17% of the TP (0 min, 0). Sulphuring caused a significant decrease in its contnet (0 min, I) probably as a result of intensive pectolytic enzymes activity (see parallel increase in SP). Dehydration of I (0 min, II) apparently did not cause further decrease in CaP, but a decrease in the CaP content was observed after storage (0 min, III).

The decrease in the CaP content after a 5 min blanch (PE inactivated; 5 min, I) indicates heat induced degradation, which is even more pronounced after dehydration (5 min, II). The amount of CaP remained stable during storage (5 min, III).

The heat induced degradation of CaP was more pronounced after 15 min blanch (I). The increase in CaP after the dehydration and storage (15 min, II, III) could be caused by natural differences in the fresh fruit or by the reaction of endogenous and exogenous Ca, with degradation products of the other fractions (see Discussion).

The PP fraction - Fig 1D - is probably the most stable and it is also interconnected with hemicellulose in the fruit tissue. It represented about 23% of the TP (0 min, 0). Sulphuring (0 min, I) caused a significant decrease in the PP. A similar content of PP was observed after dehydration (0 min, II), while the small increase observed after storage (0 min, III) was insignificant, probably because of differences in the fresh peaches. The observed increase in PP in the 5-min blanched peaches (as compered with the fresh) is probably a result of the wash -out of soluble solids and the calculation on DM basis (5 min, I). No decrease in the PP content was observed after dehydration (II) or storage (III).

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The same wash out of soluble solids to an even higher degree - would occur after 15 min blanch (see Table 1). Therefore, one has to compare the PP content of the 15-min blanched peaches with that of the 5-min blanched ones, in order to assess the heat degradation effect. A 15 min blanch caused significant degradation of the PP (15 min, I), while no further decrease in PP content was observed after dehydration (II) and storage (III).

Water-absorption capability

The effect of blanching duration on the rehydration weights (100% = weight of dry peaches before rehydration) at room temperature and on cooking is shown in Fig 2. The only sulphured (0 min) dry peaches absored about twice their weight in water. A 3-min blanch caused an increase in the rehydration weight, while the 5-min-blanched peaches absorbed about 25% more water than the non-blanched ones (see parallel increase in TP and PP). The longer time blanching times (9 and 15 min) degraded the pectins and caused a decrease in the water-absorption capacity of the peaches.

A different behaviour is observed after cooking, as compared with room rehydration, probably because of its effect on the pectins and other more stable fiber constitutents (hemicellulose, cellulose). The lower rehydration values observed following the cooking are also probably due to heat-caused disintegration of the peach tissue and faster diffusion of the water-soluble solids to the surrounding medium (water). A gradual small increase in water-absorbing capacity was observed up to a 5 min blanch, a greater increase between 5 and 9 min, and again a small increase with a 15-min blanch.

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Different behaviour, both at room and boiling temperatures (cooking) rehydration was observed in the dry peaches stored for 1 year (Fig 3), parallel with the observed changes in the TP and other pectic fractions changes during storage. In any event, the adequately (5 min) blanched peaches abosrbed more water than peaches from the other treatments. Lower water absorption was observed in the non-blanched (0 min) peaches,(active anzymes) and after 9 or 15 min blanch (probably due to the heat-induced degradation during storage). The difference between a 9 and ≥ 15 min blanch could be due to transfiguration of the pectic compounds as well as to a heat induced variation in the water absorption capacity of other CW fractions (see Discussion).

The integrity of the peach tissue, as well as the diffusion of non-bound soluble solids from it to the surrounding medium, affect also the total solids (DM) losses during rehydration. The effect of the blanching on the dry matter losses (DML) during rehydration at room temperature is given in Fig 4. About 60% losses were observed in unblanched or 3 min, blanched peaches. A significant decrease in DML was observed with the 5 min blanch (inactivation of pectolytic enzymes). An extended heat treatment, causing heat degradation of pectic substances, is probably the main reason for the observed increase in DML during rehydration of the 9- and 15-min - blanched dry peaches.

Pectins, rehydration and firmness

In order to determine whether pectins indeed play a significant role in the rehydration capacity of dried fruits, the correlation between the rehydration weight and the pectin content of the dehydrated peaches was assessed (Table 2). Significant correlation was observed between the Tp or PP content and the rehydration weight at both room

and boiling temperatures. No significant correlation (at the 95% level) was found with the CaP or SP. These results indicate that pectins play a major role in the rehydration capability of the dehydrated fruits, while PP is the major fraction affecting the rehydration.

A decrease in firmness due to heat treatments (blanching) of plant tissue is a well documented phenomena. The effect of the blanching time on firmness (compression) of the blanched nondehydrated peaches and on the parallel TP content is detailed in Table 2. A signficant correlation was found between blanching time and firmness, and between firmness and TP content of the blanched peaches. This indicates that softening of plant tissue caused by heat treatments is directly connected with degradation of pectin.

Discussion

Pectic substances are more susceptible to enzymatic or heat-induced chemical degradation than other constitutents of the CW and the IC tissue. In addition they would influence the water holding/absorbing capacity of plant tissue - forming gels at certain conditions with sugars, acids and water. The natural content, the configuration, and enzymatic or heat-induced changes in their composition, affect the structural-textural characteristics of processed plant tissue in general and of the pre-treated and dehydrated peaches in this case.

Pectolytic enzymes are responsible for degradation of pectins. Exo-PG acitivity, for example, would remove terminal galacturonic acid monomers and oligomers from the pectins' molecule, thereby reducing the content of the pectic fractions and the TP, while endo-PG attacks the linear polygalacturonic molecule of the PP (and other pectins) in a random fashion, degrading the macro-molecular structure very effectively (Pressey and Avants, 1978). Pectins' destruction should lead to a

weakening of the CW, with all the consequent changes in behaviour. The degradation of the PP complex molecule would cause also transfer of PP to SP. Reactions of SP with endogenous or exogenous (from the cooling tap-water for example) Ca, would form CaP. These phenomena affect both the firmness and the water absorption/holding capacity of the fruit tissue. Heat degradation although different from enzymatic degradation, should lead to similar effects on the textural-structural characteristics of plant tissue, as shown by Matthe and Appledorf (1978), Parrot and Thrall (1978), and Rotstein and Cornish (1978).

The products of the degradation of the different pectic fractions vary, according to the natural variability of the substrate as well as to the effects of the different enzyme - or heat-induced reactions. For example, if major pectin fractions are reduced to short water - and alcohol-soluble oligomers and monomers (due to different treatments), they do not appear in the respective contents, (Postmayer et al 1956). If the degradation is to polymers, they would form also part of other fractions, etc'. Therefore, the separated major fractions of the SP and CaP, after blanching, sulphuring, dehydration and storage, are not necessarily the same as observed in the fresh peaches. Column fractionation of each of the major pectin fractions, confirmed the above; the results are being prepared for publication.

The main degradation to monomers and oligomers was apparently caused by enzymes (0 min blanch), reducing significantly the TP and the SP (Fig. 1A,B). A short (5 min) blanch inactivated the PE without causing any significant heat-induced degradation to monomers and oligomers, and

the TP retained almost the same value as in fresh peaches (Fig 1) with a parallel high rehydration capacity (Fig 2) and lower rehydration losses (Fig 4).

Sugars, acids, GA monomers and oligomers are both alcohol- and water-soluble, and are extracted during the blanching (condensed steam, cooling water). Therefore, the relative content of the complex insoluble PP on DM increased after the blanching, both in the total solids (DM) and in the AIS. The increase in SP observed after sulphuring of the unblanched peaches is probalby a result of the enzymatic degradation of the two other fractions to SP (Fig 1B,C,D). Enzyme or heat-induced degradation of the CaP is probably the reason for the decrease in CaP content in parallel, in the unblanched (0 min) and blanched (5 or 15 min) peaches. Enzymatic degradation was probably responsible for the further decrease in the CaP content of the unblanched dehydrated peaches on storage.

The decreased rehydration capacity after longer blanching of the peaches (Fig 3) is probably caused by the heat-induced pectin degradation in the 15-min-blanched peaches, as compared with the parallel pectin content in the 5-min-blanched peaches (Fig 1A,B,D). The different behaviour during rehydration at boiling temperature (Figs 2,3) is probably due to the further heat-induced changes in the pectins as well as in other fruit tissue fiber constituents - hemicellulose and cellulose - which are affected by cooking (Matthee and Appledorf, 1978). The significant correlation found between the pectin content of the dehydrated peaches and their rehydration weight, as well as between the actual pectin content and the firmness of the non-dehydrated peaches, confirms that pectins, and specifically PP, play a major role in the structural-textural characteristics of fruit products in general and dehydrated fruit in particular.

Conclusion

Blanching (as proposed by Mrak and Perry, 1948), is not a generally practiced method for stabilization of dehydrated fruits. This work indicates that adequate blancing stabilizes one of the CW and IC tissue components, most suceptible to degradation-pectins, thereby affecting positively the rehydration capacity and reducing the rehydration losses of the dehydrated fruit. Optimum blanching, which depends on the tissue to be blanched, the size of the pieces, etc', can be recommended for stabilization of textural-structural quality characteristics during dehydration and storage of dehydrated fruits.

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Figure Legends

Fig. 1

Blanching time, sulphuring, dehydration, storage (1 year) and pectin content of dehydrated peaches (on a dry matter basis; TP of fresh untreated fruits = 2997 mg/100g dry matter).

O, Fresh (untreated); I, Sulphured only (no dehydration); II, I + dehydration; III, II + stored for 1 year; A - Total; B - Soluble; C - Ca pectate; D - Protopectin.

Fig. 2

Effect of blanching time on rehydration at room temperature (A) and cooling in boiling water (B) of sulphured dehydrated peaches (on dry matter).

Fig. 3

Rehydration capacity after 1 year storage of blanched, sulphured, dehydrated peaches (on dry matter).

Rehydration at room temperature, circles; Rehydration in boiling water for 30 min, triangles.

Fig. 4

Dry matter losses during rehydration at room temperature of blanched, sulphured, dehydrated peaches (weight of dehydrated peaches = 100%).

Table 1 - Effect of blanching and sulphuring on the dry matter (DM) and alcohol insoluble solids (AIS) contents of peaches.

Blanching time* (min)	Dry matter %	Dry matter ratio***	Alcohol insoluble solids** %	Alcohol insoluble solids** ratio***
0 (fresh fruit)	17.0	1.00	13.2	1.00
5	15.2	0.89	15.9	1.20
15	14.2	0.835	15.4	1.17

* Before sulphuring

** On dry matter basis

*** Calculated on the value of the fresh peach halves = 1.0

Table 2 - Rehydration weight (%) and pectin content (mg/100g dry peaches) of sulphured dehydrated peaches

Blanching time (min)	Rehydration weight (%)		1) Total pectin (mg/100g)	2) Protopectin (mg/100g)
	a) Room*	b) Boiling**		
0 min - A++	277+	-	1780	450
15min - A++	294+	218	2428	512
15min - B++	314+	220	2482	593
5 min - A++	330+	-	2628	731
5 min - B++	347+	250	2781	1135
	(a) and (1)	(a) and (2)	(b) and (1)	(b) and (2)
r	0.9097	0.9282	0.9975	0.9979
p	$0.01<p<0.05$	$0.01<p<0.05$	0.05	0.05

* After 24 hr in water at room temperature

** After 30 min. in boiling water.

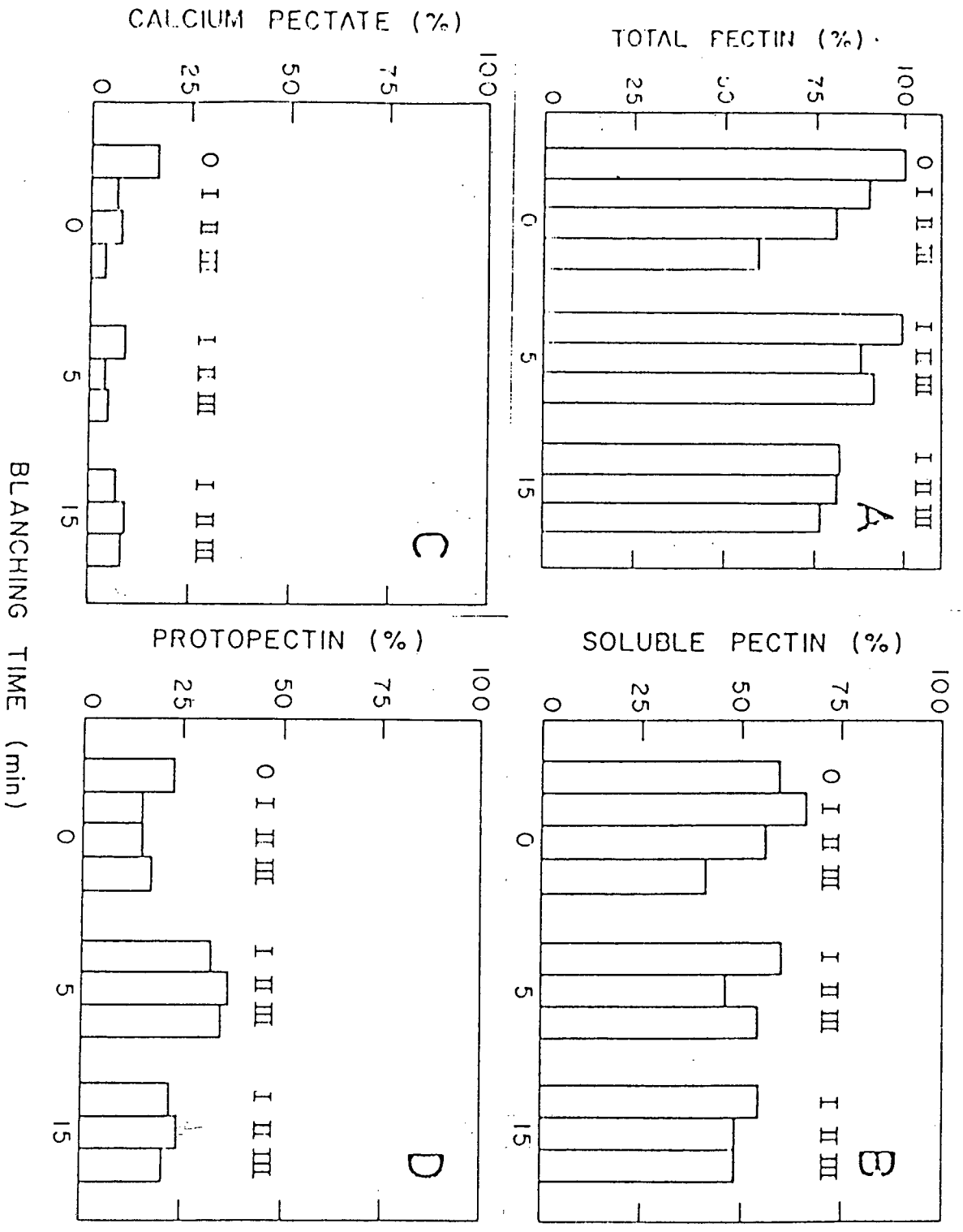
\+ Weight of non-rehydrated fruits 100%

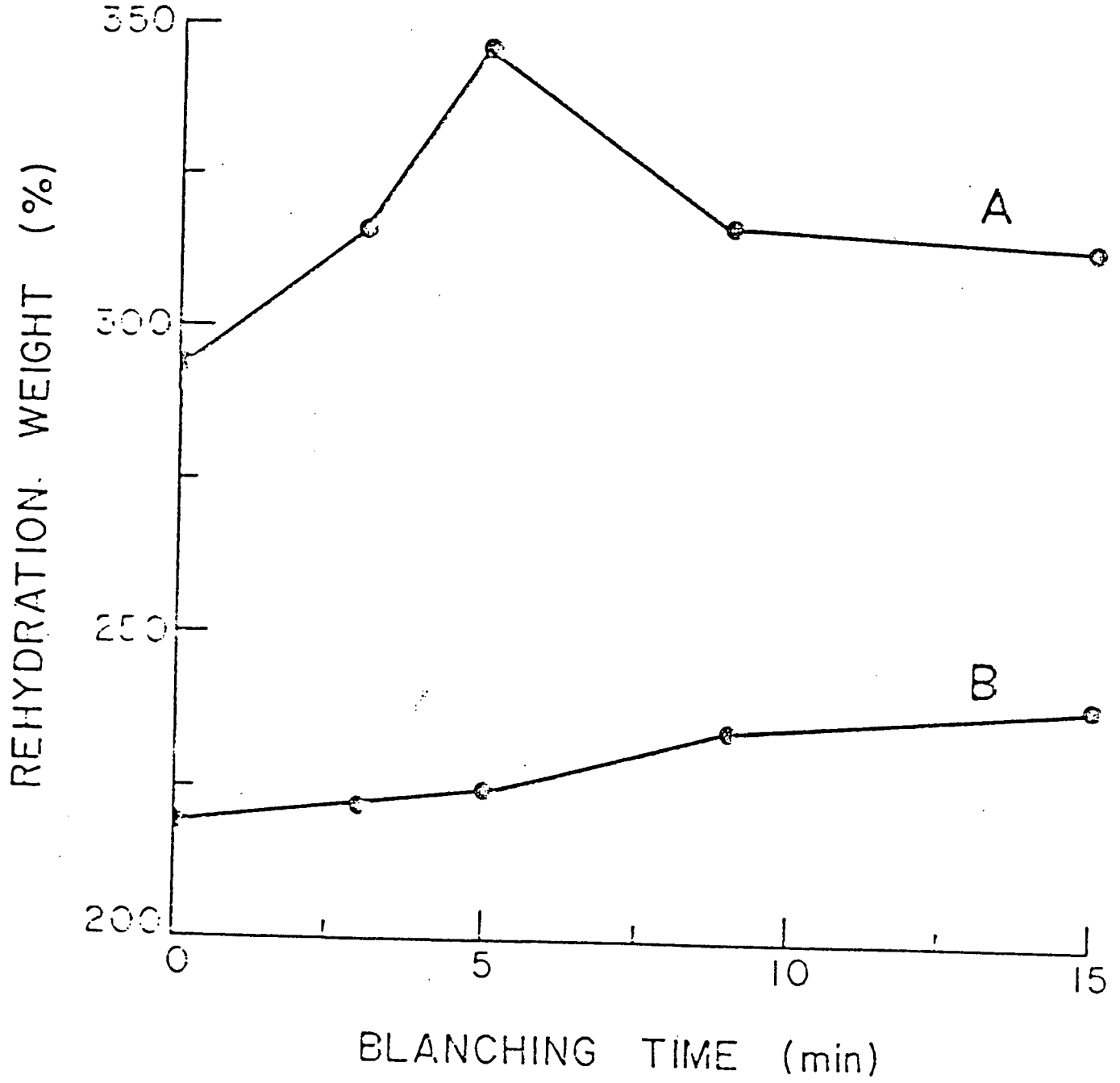
++ A and B - samples from different batches of peaches.

Table 3 - Pectin degradation and firmness of blanched sulphured peaches

a) Blanching time (min)	b) Firmness (Newtons)	1) Total Pectin* (mg/100g fruit tissue)
0	21.6	510
5	15.5	454
15	7.8	349
	(a) and (b)	(b) and (1)
r	0.9992	0.9984
p	0.05	0.05

* Actual content in non dryed peaches.


TOTAL PECTIN (%)
CALCIUM PECTATE (%)
SOLUBLE PECTIN (%)
PROTOPECTIN (%)
0
25
50
75
100
0
5
15
I
II
III
A
B
C
D
BLANCHING TIME (min)


350
300
250
200
REHYDRATION WEIGHT (%)
A
B
0
5
10
15
BLANCHING TIME (min)

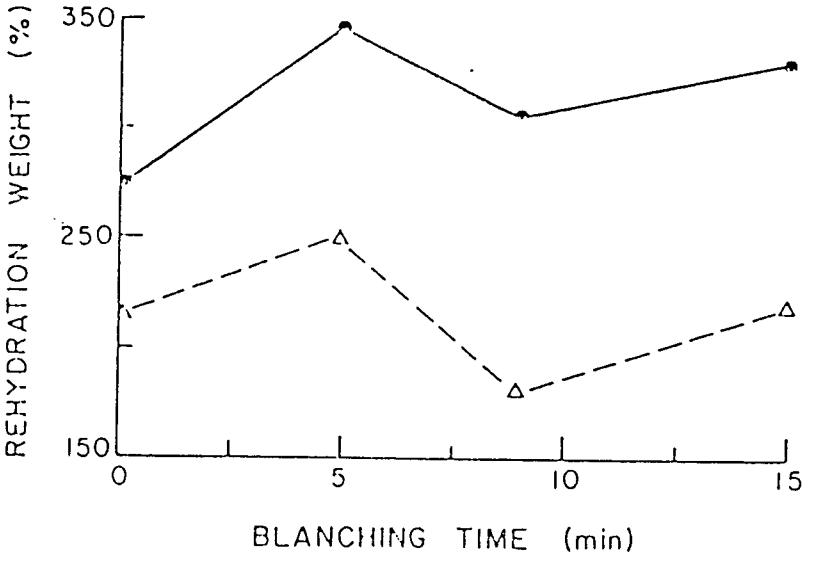

REHYDRATION WEIGHT (%)
350
250
150
0
5
10
15
BLANCHING TIME (min)

Fig. 4