4

BARD

FINAL REPORT
PROJECT NO. US-452-81

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

D.S. Reid, B.S. Luh, A. Levi, N. Ben-Shalom

28

630.72
BAR/RPI
2nd copy

1987

000000011709

24419

24419

20449 70

Standard BARD Cover Page For Scientific Reports date April 1987

BARD

P.O.B. 6

Bet Dagan 50250 ISRAEL

BARD Research Project No US-452-B1

Title of Research Project:

Factors influencing the quality characteristics of frozen and dehydrated fruits and vegetables.

Name and Address of Investigators

Principal Investigator: D.S. Reid

Cooperating Investigators: B. Luh

A. Levi

N. Ben Shalom

Name and Address of Affiliated Institutions

Principal Institution: U.C. Davis, Davis, CA USA

Cooperating Institution: Volcani Institute, Bet Dagan, Israel.

Project Commencement Date.....

Type of Report: 1st Annual.... 2nd Annual.... Final X..

Signature

Principle Investigator

Institution's Authorized Official
(signature and official stamp)

David S. Reid

Allen E. Marr

Allen E. Marr, Deen (916) 752-2075
Graduate Student and Research
Officer, 277 Mark Hall
University of California, Davis
Davis, California 95616

RECEIVED 10 2075
MARR, ALLEN E.
107-012

630.72
BAR / REI
2nd copy

: 664.802.85; 664.802.84;
574.192.94; 581.192.94;
641.1

Contents

1. Abstract
2. Introduction
3. Methods
4. Results and Discussion
5. Conclusions
6. Cooperation
7. Appendices

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 prepared¹. 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 tissues³. 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 machine⁴. 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 content², 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 change in the tissues are then followed by specific studies of the effects of processing on the pectic fractions which can be 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 tissues 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

1. A.E.R. Ahmed and J. Labavitch, J. Food Biochem 1 361, (1977).
2. R.M. McCready and E.A. McComb, Anal Chem 24, 1630,.
3. P. Baardseth, Food Chem.,3, 271, (1978).
4. M.C. Bourne and J.C. Moyer, Food Technol., 22, 1013, (1968).
5. N. Blumenkrantz and G. Asboe-Hansen, Anal. Biochem.,54, 484, (1973).
6. P. Albersheim, D.J. Nevins, P.D. English and A. Karr, Carbohydrate Res. 5, 340, (1967).

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 lipxygenase

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 3

(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 occurring, 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*.

... from three different

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 blanchd at 212°F, unfrozen for green beans.

Pectic fractions of blast frozen peaches stored at -20

Fraction	Unfrozen		1 Day		4 Months	
	$\mu\text{g GA}/\mu\text{g CWAIS}$	%	$\mu\text{g GA}/\mu\text{g CWAIS}$	%	$\mu\text{g GA}/\mu\text{g CWAIS}$	%
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 matter).

TREATMENTS	SOLUBLE PECTIN	%	CALCIUM 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	%
<u>UNTREATED TISSUE</u>								
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
<u>BLANCHED TISSUE</u>								
<u>PH-6.3</u>								
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
<u>BLANCHED DEHYDRATED</u>								
<u>TISSUE</u>								
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
<u>DEHYDRATED</u>								
<u>TISSUE</u>								
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)						Total Sugars	
	Fraction No.							
	A	%	B	%	C	%	ug	%
<u>Untreated tissue</u>								
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
<u>Blanched tissue</u>								
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	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

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.

<u>Sample</u>	<u>Percent D.E.</u>
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 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.