THE UNIVERSITY OF MANITOBA

FACTORS INFLUENCING SUCROSE INVERSION AND EXTRACTION IN *RUBUS IDAEUS*

by

GRANT ANDREW RIGBY

A Thesis

Submitted to the Faculty of Graduate Studies

In Partial Fulfillment of the Requirements for the

Degree of Master of Science

Department of Food Science

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ABSTRACT

The effect of storage and pasteurization on sucrose inversion into fructose and glucose in Rubus idaeus L., Boyne cultivar, was investigated using high performance liquid chromatography. A slow rate of sucrose inversion was observed to occur in the whole fresh fruit when held at 4°C. Freezing and thawing, or crushing, hastened the rate of sucrose inversion at 23°C. Pasteurization at 80°C for thirty seconds prevented further sucrose inversion, suggesting the probable presence of invertase enzyme of microbial or plant origin.

I. INTRODUCTION

This research investigated processing and quality aspects of clarified juice from fruit of the red raspberry (Rubus idaeus) grown on the Canadian Prairies.

A. PRAIRIE RASPBERRY INDUSTRY

The possibility of producing red raspberries on the Canadian Prairies has been long evidenced by the crop's success in home gardens as a source of fresh fruit. The commercial red raspberry production industry on the Prairies has grown to occupy approximately 107 hectares in total, and is primarily dependant upon selling unprocessed fresh or frozen fruit directly to the consumer (Western Canadian Society for Horticulture, 1989). Raspberries are typically consumed fresh in the harvest season as a dessert, or at other times in the form of home preserved jams. The primary mode of product delivery to the consumer is via U-pick marketing, in which the consumer manually harvests the raspberries which he or she then purchases. U-pick marketing can gross the grower typically $2.20 to $3.60 per kilogram of raspberries with no harvesting costs. This price compares very favourably with the market value for processing raspberries, which in 1987, 1988 and 1989 was at approximately $1.21 to $1.37 per kilogram to the British Columbia Frazer Valley growers, before deducting their costs of harvesting. Increases in the planted area in the states of Washington and Oregon, and

relatively large North American frozen inventories prior to the 1990 harvest, theatened to further reduce prices received by growers for processing raspberries (Schmidt, 1990). Established Prairie raspberry growers who have successfully targeted local high value fresh markets frequently produce raspberries in surplus to that which can be sold; a U-pick grower, for example, may have an unexpectedly large crop or may not be able to attract sufficient customers due to inclement weather. For these growers it may be worthwhile to salvage the surplus production by harvesting it for sale to the lower value processing market. The establishment of a local small scale processing market would meet their irregular requirements for marketing the surplus production, and thereby function as an indirect support of price levels in the fresh market.

Since released in 1960 by the Agriculture Canada Research Station at Morden, Manitoba, the cultivar "Boyne" has become the favoured cultivar on the Prairies, exhibiting improved winter hardiness and yield over older cultivars. Lazzari and McConnell (1985) provided evidence of Boyne raspberry's potential suitability for processing, having quality attributes similar to those of the major British Columbia cultivars which are utilized for yogurt purees, jams, and juices. There remains uncertainty, however, with regards to the cost competitiveness of Prairie production of processing raspberries when compared with production costs in British Columbia, Washington and Oregon. The major production problems are related to the harshness and variability of the Canadian Prairie climate in comparison with the milder climates where raspberries are long established crops grown successfully for

processing. R. Braun, A. Arnott, L. Williams, G. Little, F. Dreidger, W. Thiesen, R. Beavis, R. Hicks, M. Ganczar, R. Martens, G. Rigby and others (personal communications, 1986-1990) have experienced severe problems relating to several climatic factors. These factors include extreme heat and wind desiccating the fruit, accelerating spider mite population increases, and excessively warming organic soils, extreme cold fluctuating with mild winter temperatures resulting in winter dieback of primocanes, deep snow drifts breaking down canes during the spring thaw, late spring frosts destroying early flower buds, atypical periods of excess moisture and fog resulting in anthracnose, spur blight and cane blight fungal diseases, and hail storms devastating both the current and subsequent year's crops. Some regions of the Prairies experience less severe climatic extremes than other regions. The localized moderation of climate by Lakes Manitoba and Winnipeg (Dunlop and Shaykewich, 1982, and I. Wishart, personal communication, 1988) is one such example, as are north-east facing mid-slope positions in rolling semi-wooded areas, which are relatively protected from late spring frosts and hot south winds and assured of adequate water drainage under all conditions. The Prairies may be favoured for raspberry production by its typically lower relative humidities compared with the west coast production which may lessen problems with fungal diseases. Most regions of the Prairies also have relatively low priced land compared with the approximate price of $7,000 per hectare in northern Washington (G. Pinton, B.C. Raspberry Growers Assoc., personal communication, 1990). The fact that raspberries do not require stone-free or level land suggests that as an enterprise option the crop may be

better able to compete for land utilization in areas which are unsuitable for high value vegetable crops.

B. RESEARCH OBJECTIVES

The probable suitability of Boyne cultivar raspberry for processing (Lazzari and McConnell, 1985), combined with the yearly availability of a small quantity of Prairie grown Boyne raspberries surplus to the fresh market demand, as well as a general interest amongst local growers in modestly expanding raspberry production (J. Portree, personal communication, 1985), led to the decision to investigate raspberry processing. Recent advances in fruit juice processing technology, especially the advent of ultrafiltration as a process operation for the production of clarified juices (Moslang, 1984, Pepper, 1987, Swientek, 1986, Breslau et al., 1984, Kirk et al., 1983, Wilson and Burns, 1983, Cheryan, 1986), invited the question of its potential utility for the processing of a raspberry juice. Extensive searches of the scientific literature and communications with equipment suppliers yielded no reports of ultrafiltration being used in raspberry juice processing prior to early 1987 when first conducted as part of this research study.

The overall research objective was to develop a small scale processing system using locally available equipment for the commercial extraction and clarification of raspberry juice. There were three phases to this research:

1. Laboratory investigation of raspberry juice extraction. This work established some of the key parameters for the subsequent pilot scale trials.

2. a. Pilot scale development of an extraction and ultrafiltration clarification process system. Due to the high cost of raspberries, and of equipment and space rental, much of this work could not be replicated with complete control of all external variables and is reported here as a demonstration only.

b. Analysis of quality of the clarified raspberry juice extract processed, including comparison with a commercial product and comparative sensory evaluation of a formulated juice beverage, to assess the potential suitability of the juice extract as a primary ingredient for commercial juice beverages.

3. Investigation of some parameters influencing the hydrolysis of the native sucrose in raspberry into glucose and fructose. This work sought to explain the difference between the absence of sucrose in the clarified Boyne raspberry juice extract processed here, and the high level of sucrose identified by Lazzari (1986) in a juice extract of fresh Boyne raspberries.

II. LITERATURE REVIEW

A. FRUIT STRUCTURE AND COMPOSITION

1. RASPBERRY FRUIT STRUCTURE

The raspberry fruit is an aggregate of drupelets, each drupelet developing entirely from a single ovary (Figure 1)(Reeve, 1954a and 1954b). The whole berry thus develops from a number of ovaries from the same flower, all adhering to a common receptacle (Jennings, 1988, Esau, 1977). Unicellular epidermal hairs on the surface of each drupelet entangle with those of adjacent druplets to hold the berry together. Below the epidermal layer is a hypodermis of slightly collenchymatous cells. Internally, the succulent mesocarp of the drupelet consists of turgid thin-walled parenchymatous cells. In the centre is a pyrene with a hard endocarp enclosing the seed.

2. RASPBERRY FRUIT COMPOSITION

a. Overall composition of raspberry (Table 1). Lazzari and McConnell (1985) analysed previously frozen and then thawed and pureed whole Boyne raspberries, produced during 1983 and 1984 at Morden and McCreary, Manitoba. Total solids in the whole fruit were found to range from 13.5% to 15.1%, and total soluble solids in the whole fruit to range from 9.5% to 11.3%. The difference between total solids and total soluble solids represents total insoluble solids, which were calculated to range from 3.8% to 4.3% of the whole fruit. The theoretical maximum

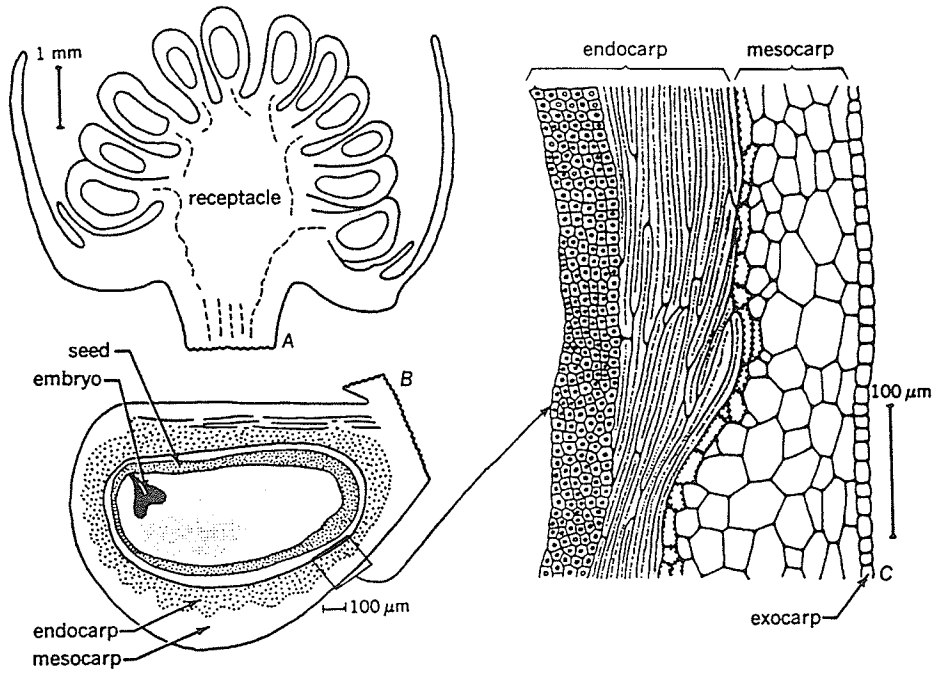


FIGURE 1. DIAGRAM OF IMMATURE FRUIT OF RASPBERRY. A. LONGITUDINAL SECTION OF ENTIRE IMMATURE FRUIT SHOWING AGGREGATION OF DRUPELETS ON RECEPTACLE. B. LONGITUDINAL SECTION OF A SINGLE IMMATURE DRUPELET. C. LONGITUDINAL SECTION OF A FRAGMENT OF DRUPELET PERICARP, SHOWING MESOCARP PARENCHYMA WHICH SWELLS TO BECOME SUCCULENT FRUIT TISSUE. FROM REEVE (1954) AND ESAU (1977).

TABLE 1. COMPOSITION OF BOYNE RASPBERRY GROWN AT MORDEN AND McCREARY, MANITOBA. ADAPTED FROM LAZZARI AND McCONNELL (1985).

Component	1983 Crop	1984 Crop
Morden:		
Whole fruit:		
Total Solids	13.47% +/- 0.45*	14.42% +/- 0.76*
Soluble Solids	9.54% +/- 0.11	10.16% +/- 0.30
Insoluble Solids**	3.93%	4.26%
Filtered juice extract:		
Soluble Solids	8.63% +/- 0.08	9.13% +/- 0.24
McCreary:		
Whole fruit:		
Total Solids	14.88% +/- 0.17	15.12% +/- 0.93
Soluble Solids	10.87% +/- 0.16	11.31% +/- 0.58
Insoluble Solids	4.01%	3.81%
Filtered juice extract:		
Soluble Solids	9.69% +/- 0.12	10.05% +/- 0.46

Notes:
* Means +/- standard deviation (n=3).
** Insoluble solids = total solids - soluble solids.

recoverable juice extract was thus 96.2% to 95.7% of the whole raspberry, assuming that it is technically possible to remove only the insoluble solids fraction, the rest being water and solubles. Soluble solids in the filtered juice extract were determined by refractometer and ranged from 8.6% to 10.1%.

b. Sugars. Lazzari (unpublished data, 1986), based upon high performance liquid chromatography (HPLC) analyses of Boyne raspberries from the 1983 and 1984 Morden, Manitoba, crops, found that as percent of total major sugars in a pressed juice extract of fresh whole berries, fructose accounted for 31.7% and 32.6% respectively, glucose for 23.7% and 27.4%, sucrose for 43.2% and 38.7%, and galactose for 1.4% and 1.3% (Table 2). Based upon compilations from six literature sources, Wrolstad and Shallenberger (1981) reported fructose levels to range from 20.2% to 48.3%, glucose from 19.8% to 43.4%, and sucrose from 8.3% to 51.8%. Green (1971) reported fructose and glucose to be the dominant sugars in mature raspberry fruit, with sucrose present at only 0.06% when the total sugar level was 1.57%, and present at 1.21% when the total sugar level was at 5.34%. Benk and Bergmann (1977) reported fructose to range from 24.2% to 48.8%, glucose from 20.8% to 44.8% and sucrose from 0% to 12.6%. Bettenfeld and Voilley (1983) found glucose at 1.90%, fructose at 1.79%, and very little sucrose in thawed raspberry puree. There is thus considerable variability in the reported total sugar content in raspberries and in the specific contribution of sucrose to the total. This variability might reflect genetic differences between cultivars, different climates or production systems, different

TABLE 2. INVERT SUGAR LEVEL IN RASPBERRY. COMPILATION FROM THE LITERATURE.

Invert Sugar* (% of total sugars)	Origin	Analysis Method	Reference**
56.2	Man. (83 Boyne)	HPLC	L.
60.8	Man. (84 Boyne)	HPLC	L.
50.5	U.S.A.	paper chromatography	W.,S.
48.2	U.S.A.	paper chromatography	W.,S.
52.0	U.S.A.	paper chromatography	W.,S.
71.7	Switz.	enzymic	W.,S.
91.7	Germany	enzymic	W.,S.
82.9	England	chemical	W.,S.

Notes:
* Invert sugar % = ((fructose + glucose)/(fru. + glu. + sucrose)) x 100
** References: L. = Lazzari (unpublished data, 1986)
W.,S. = Wrolstad and Shallenberg (1981)

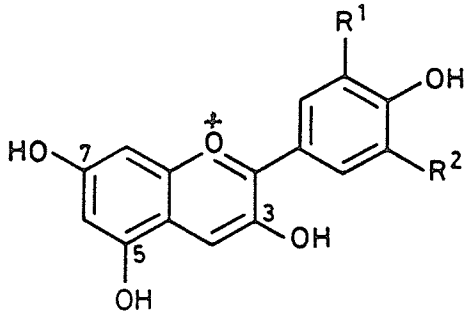
stages of maturity at harvest, or different treatment conditions prior to analysis. The potential for hydrolysis of sucrose into glucose and fructose prior to analysis should be considered. Such hydrolysis (also known as "inversion") could be catalysed by wild yeast origin extracellular invertase enzyme (Reed and Underkofler, 1966, and Weger, 1978). Acid conditions have also been shown to catalyse sucrose inversion (Wienen and Shallenberger, 1988). Sucrose, fructose and glucose each impart different sweetness intensities (Potter, 1978), and thus the percieved sweetness of a raspberry juice extract would vary with the relative and total concentrations of the three sugars.

c. Acids. Lazzari (1986) found citric acid be the dominant organic acid in Boyne raspberry juice extract from the 1983 and 1984 Manitoba crops, constituting 74.4% and 88.3%, respectively, of the total organic acids, with malic acid constituting 16.7% and 6.4%, respectively, and succinic acid constituting 8.9% and 5.4%, respectively. Green (1971) also reported the main acid present in red raspberries to be citric acid, together with a small amount of malic acid. Ryan and Dupont (1973) reported citric acid to constitute 97.4% of the total acids in raspberry juice. Acids contribute the sensory attribute of sourness, the ratio of sugars to acids being an important predictor of perceived sweetness or sourness (D'Souza, 1986, Potter, 1978).

d. Colours. The red colour of raspberry is due to the presence of anthocyanins. Anthocyanins in raspberry are glycosides comprised of an

anthocyanidin and one to three carbohydrate units. Cyanidin and pelargonidin (Figure 2) are the anthocyanidins found in raspberry (Coultate 1989, Jennings 1988), with pelargonidin occuring only in trace amounts (Jennings and Carmichael, 1980). Glycosylation occurs at position 3 of the anthocyanidin, with six structures having been identified in raspberry (Figure 3), (Jennings and Carmichael, 1980). Glycosylation is thought to contribute to the stability of the pigment, when compared with the corresponding free anthocyanidin (Mazza and Brouillard, 1987). Increasing the pH of a raspberry juice extract results in a decrease in the proportion of the anthocyanins which are present in the red coloured flavilium cation form, and an increase in the proportion which are in colourless or blue forms (Coultate 1989, Mazza and Brouillard, 1987). Heating an anthocyanin solution results in an increase in the proportion of the anthocyanins which are in the colourless chalcone form, but this may be reversible as the solution is cooled (Mazza and Brouillard, 1987, Brouillard, 1982).

e. Aroma compounds. Major volatile compounds contributing to the characteristic aroma of raspberry have been isolated from the cultivar "Lloyd George" by Guichard (1984), Guichard and Issanchou (1983), and Guichard (1982). Many ketones, alcohols, terpenes and esters were reported to be present in a nitrogen swept headspace above previously frozen raspberries. The extraction methodology used affected the relative concentrations of volatile components determined by subsequent gas chromatography and mass spectrophotometry. A method combining nitrogen entrainment with trapping in an ethanol solution and then



	R^1	R^2
pelargonidin	—H	—H
cyanidin	—OH	—H

FIGURE 2. CHEMICAL STRUCTURES OF CYANIDIN AND PELARGONIDIN. FROM COULTATE (1989).

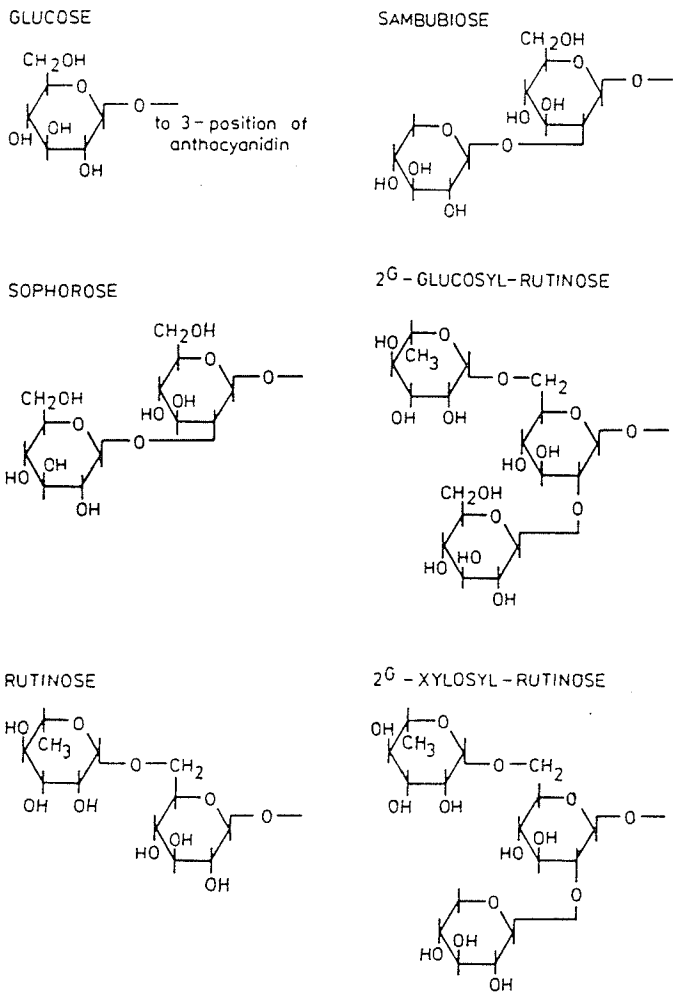


FIGURE 3. CHEMICAL STRUCTURES OF GLYCOSYLATION OF ANTHOCYANINS. FROM JENNINGS AND CARMICHAEL (1980).

liquid:liquid extraction with Freon 11, preferentially isolated ketones and alcohols when compared to a method combining nitrogen entrainment with trapping on Chromosorb 105 adsorbant and elution with dichloromethane (Table 3)(Guichard, 1984). The ketones alpha-ionone, beta-ionone (Figure 4), dihydro-alpha-ionone and dihydro-beta-ionone are considered by some researchers to be the most important compounds of the genuine raspberry aroma (Guichard and Issanchou, 1983; Hiirsalmi et al., 1974; Latrasse, 1982). The compound commonly known as "raspberry ketone", 1-(4'-hydroxy-phenyl)-3-butanone (Figure 5) was not identified as being present in raspberry in the Guichard work on volatiles. In a separate study motivated by the need to develop procedures to detect adulteration of raspberry products by quantitatively determining if chemically synthesised "raspberry ketone" had been added, Gallois (1982) reported the compound to be naturally present in 22 cultivars of raspberry at levels ranging from 20 to 370 micrograms per 100 grams of fruit, with the highest level being found in the cultivar Lloyd George. Fogy et al. (1981) similarly reported "raspberry ketone" to be present at concentrations less the 200 micrograms per 100 grams of genuine raspberry fruit, and noted that very much higher levels of the compound, for example up to 350,000 micrograms per 100 grams, were detectable in artificially flavoured imitation raspberry products. The general scientific literature appears to be inconsistent with respect to the role of "raspberry ketone" in genuine raspberry aroma. Coultate (1989) agrees that the compound has been used as the major component in artificial raspberry flavourings, but also states that the distinctive character of raspberry aroma is due mostly to this compound, which

TABLE 3. VOLATILE AROMA COMPOUNDS IN RASPBERRY. ADAPTED FROM GUICHARD (1984).

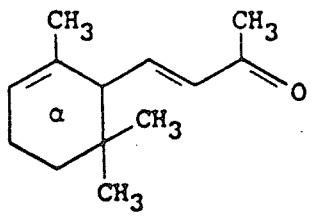
Compound identified	Nitrogen entrainment / adsorbent trapping	Nitrogen entrainment / solvent extraction	Character aroma **
KETONES:			
2-Heptanone	938 *	960 *	peppery
2-Nonanone	81	56	
Piperitone	2	10	peppermint
2-Decanone	0	6	
Dihydro-α-ionone	24	47	
α-Ionone	129	332	cedarwood, violet
Dihydro-β-ionone	45	102	
β-Ionone	125	363	
% Ketones	13.4%	18.8%	
ALCOHOLS:			
1-Hexanol	170	801	
2-Heptanol	16	70	
Linalool	306	1246	lavender
Terpinen-4-ol	60	241	
α-Terpineol	10	77	
Nerol	12	87	sweet rose
Geraniol	54	301	sweet rose
% Alcohols	6.3%	28.2%	

Notes:
* values indicate relative concentration in the extract.
** The Merck Index (1983).

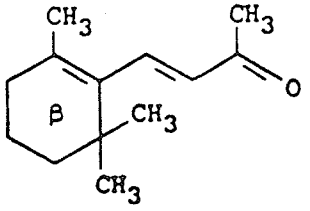
TABLE 3. (cont.) VOLATILE AROMA COMPOUNDS IN RASPBERRY. ADAPTED FROM GUICHARD (1984).

Compound identified	Nitrogen entrainment / adsorbent trapping	Nitrogen entrainment / solvent extraction	Character aroma **
TERPENES:			
α-pinene	3529 *	1742 *	turpentine
β-pinene	154	73	
Myrcene	435	240	
α-phellandrene	1634	1195	bitter fennel
ρ-cymene	315	158	
β-phellandrene	1016	622	
γ-terpinene	107	135	
Terpinolene	19	139	
Caryophyllene	249	167	cloves, turpentine
Humulene	13	67	
% Terpenes	74.7%	45.4%	
ESTERS:			
Isopentenyle acetate	193	190	
Cis-3-hexenyle acetate	58	57	
Menthyl acetate	52	196	floral, rose
% Esters	3.0%	4.4%	

Notes:
* values indicate relative concentration in the extract
** The Merck Index (1983)



Alpha-ionone



Beta-ionone

FIGURE 4. CHEMICAL STRUCTURES OF MAJOR KETONES IN RASPBERRY AROMA. FROM THE MERCK INDEX (1983).

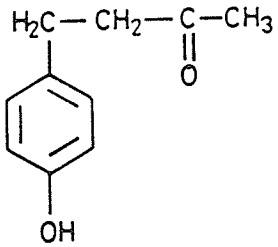


FIGURE 5. CHEMICAL STRUCTURE OF "RASPBERRY KETONE". FROM COULTATE (1989).

appears to be in disagreement with Guichard and Issanchou, (1983), Hiirsalmi et al., (1974) and Latrasse, (1982).

Process operations which avoid or minimize heating of raspberry under conditions of an open headspace would be expected to minimize losses of these relatively volatile compounds which are important components of the characteristic raspberry aroma.

B. JUICE PROCESSING TECHNOLOGY

1. OVERVIEW OF JUICE PROCESSING TECHNOLOGY

Three principle process operations are generally required for the processing of clarified fruit juice products:

a. Tissue destruction and cell rupture. Process options for tissue destruction and cell rupturing include:

i) direct physical force such as crushing;

ii) heating to induce cell rupture as a consequence of increased turgor pressure within individual cells; and

iii) freezing to induce the formation of water crystals, effecting a piercing of cell membranes.

b. Separation of a liquid extract from the solids. Liquid-solids separation can be achieved by the process operations of:

i) screening, which achieves insoluble solids removal on the basis of particle size;

ii) pressure filtration through essentially inert diatomaceous earth, similarly removing insoluble solids based upon particle size and conformation differences;

iii) undisturbed settling followed by decantation, which achieves solids removal based upon differences in the density of insoluble solids and of the liquid extract; and

iv) centrifugation, which accentuates and hastens separation based upon differences in density.

c. Clarification of the liquid extract. For the production of a clarified juice, the final objective is to obtain a stable liquid product comprised primarily of water and dissolved compounds, which is free of precipitate and is translucent to light with minimal scattering of light. In physical terms, Malcolmsom et al. (1989) interpreted the sensory attribute of clarity to relate to the transmission of light. The greater the amount of light transmitted, the more translucent or clarified is the juice. When light scattering occurs, however, due to particles in the juice, the sensory attribute of turbidity is perceived. Wucherpfennig et al. (1987) ascribe this turbidity to the presence of insoluble particles greater than one micron in size, and distinguish turbidity from the opalescence effect of colloidally soluble macromolecules. The insoluble particles which cause turbidity may include pulp particles, insoluble proteins and protein-tannin compounds, live and dead microorganisms, and salt compounds of low solubility in crystalline form.

The dissolved and colloidal macromolecules which are commonly present in fruit juices were reported by Wucherpfennig et al. (1987) to include the following polysaccharides:

-cellulose (insoluble),

-hemicelluloses, eg. xyloglucan, araban, galactans, arabinogalactan, mannan, galactomannan, glucomannan,

-pectin,

-starch, eg. amylose, amylopectin,

-yeast origin glucans and mannans,

-Botrytis origin glucans;

and other proteins, glycoproteins and polyphenolics.

Of these colloidal substances, pectin is the most important impediment of filterability of non-clarified juice. The high molecular weight of pectins together with their complex structure, consisting of neutral and acidic saccharides in linear and branched conformations, results in a tendency to form large three dimensional stable gels in solution, decreasing viscosity and binding water, solutes and other colloidal substances and insoluble particles (Coultate, 1989). The use of fungal origin pectinase-type enzymes for the hydrolysis of pectins and consequent prevention of the formation of colloidal gels, or for the destabilization of those gels already formed, is common practice in fruit juice processing (Reed and Underkofler, 1966).

Process options for clarification of the liquid extract include:

i) enzymatic hydrolysis of pectins with pectinase-type enzymes (Coultate, 1989);

ii) co-precipitation of other colloids by the addition of gelatin or bentonite; gelatin promotes the precipitation of colloidal tannins and polyphenols, and bentonite promotes the precipitation of proteins including added gelatin; and

iii) ultrafiltration to remove colloidal molecules and insoluble substances greater in molecular size than the molecular weight cut off of the membrane used.

2. NON-CLARIFIED RASPBERRY JUICE EXTRACTION TECHNOLOGY

Pederson and Beattie (1943) investigated raspberry juice processing using a rotary screen press. This consisted of forcing fresh raspberries through a series of sequentially smaller screens utilizing a screw mechanism. The greatest yield was obtained when the extraction was conducted at 49°C, but a product judged to be of better quality was obtained when the extraction was conducted on unheated chilled fruit. A turbid pulpy juice was produced, which after deaeration and pasteurization developed a foamy layer on the surface and a heavy sediment at the bottom, with a clear layer in between. Direct juice extraction from fresh raspberries through press cloth in a traditional fruit press was also investigated by Pederson and Beattie (1943). This produced a less pulpy product than with the forced screening method, but yields and colour extraction were judged to be unsatisfactory when the fruit was pressed cold, and flavor was impaired when the fruit was heated to 49°C or higher. The highest juice extraction obtained was 68%

of the total weight at 82°C. Using the same pressing method on raspberries which had been frozen with 10% sugar, stored at -18°C and then thawed, an extraction rate in the range of 65% to 75% was achieved without heating, and the colour extraction was considered acceptable. Both freezing and heating were thought to effect a reduction in the mucilaginous character of fresh raspberries, thereby facilitating juice extraction by pressing (Nelson and Tressler, 1980).

3. CLARIFIED FRUIT JUICE PROCESSING TECHNOLOGY

a. Traditional clarified juice technology. Moslang (1984) described traditional technology for the production of clarified apple juice to include the following process steps:

i) crushing;

ii) pressing;

iii) aroma stripping (to avoid aroma losses during subsequent evaporative concentration);

iv) addition of gelatin;

v) addition of pectin and starch degrading enzymes;

vi) hold period of 2 to 25 hours for reaction and precipitation;

vii) centrifugation or decantation;

viii) pressure filtration through diatomaceous earth.

Gelatin fining serves the function of removal of unstable phenolics (Cheryan, 1986). Pectinases hydrolyse insoluble pectin, preventing the formation of pectin gels, destabilizing colloidal suspensions, and reducing the viscosity of the juice (Coultate, 1989). Other treatments commonly used include bentonite fining for the coprecipitation of

unstable proteins and protein-phenolic complexes, and high-temperature-short-time (HTST) pasteurization early in the processing sequence to inactivate native polyphenol oxidase enzymes (PPO) and reduce microbial levels (Heatherbell, 1984). Early pasteurization and aseptic handling conditions would be necessary to prevent microbial growth during the extended hold period required for enzyme reaction and precipitation.

b. Traditional extraction combined with sequential filtration technology. D'Souza (1986) was able to achieve clarification and microbial sterility of Manitoba grown apple juice on a laboratory scale basis with the use of perpendicular flow non-reusable filters. This process system involves the following steps:

i) pressing;

ii) addition of pectinase-type enzymes;

iii) settling overnight;

iv) decanting of supernatant (the proportion lost as precipitate was not indicated);

v) several sequentially tighter filtrations down to a particle size of 0.22 micron, achieving clarity by removal of turbidity and microorganisms.

Filter replacement might be expensive and time consuming for scaled-up process operations; D'Sousa (1986) suggested that tangential flow ultrafiltration should be considered as an alternative.

c. Traditional extraction combined with ultrafiltration technology. Cheryan (1986) described ultrafiltration of juice as a

pressure driven separation process whereby particles, suspended solids and colloidal compounds are rejected by a semi-permeable membrane primarily on the basis of their size. Compounds which are of a lower molecular weight than the "molecular weight cut off" (MWCO) characterisitic of the ultrafiltration membrane are able to permeate through the membrane. To avoid the formation of a compacted layer of particles, suspended solids and large compounds on one side of the membrane, leading to a reduction in "flux" (rate of permeation through the membrane), the feed stream flows tangentially to the membrane surface. The membrane surface is thereby continually swept clean of those substances which are too large to interact with the membrane surface pores. The feed is thus separated into two liquid streams: the "retentate" which is retained by the membrane and contains the large substances; and the "permeate" which permeates through the membrane and is free of large substances. Most ultrafiltration systems operate in a batch mode and continually recycle the retentate back to the feed tank until the minimum hold-up capacity is reached. The permeate is typically a clarified product, the substances responsible for turbidity having been retained by the ultrafiltration membrane. Depending upon the ultrafiltration membrane employed, the permeate typically contains the relatively small compounds in a concentration approximately equivalent to that in the feed, including the sugars, acids, colours and aroma compounds.

Swientek (1986), Kirk et al. (1983), Wilson and Burns (1983) and Cheryan (1986) have indicated some of the successful juice processing applications for ultrafiltration operations, for example:

i. replacing gelatin and bentonite fining for removing colloidal compounds, and reducing pectinase enzyme requirements related to destabilizing colloids;

ii. replacing thermal treatments for inactivating enzymes such as PPO by instead retaining them in the retentate fraction;

iii. replacing diatomaceous earth filtration, and centrifugation, for removing particles and precipitates responsible for turbidity;

iv. and replacing thermal treatments for destroying microorganisms by instead retaining them in the retentate fraction.

Rigby (unpublished data, 1988) utilized the process operations of crushing, ascorbic acid addition to restrict early oxidative browning (DeMan, 1980, Coultate, 1989), pectinase reaction, juice extraction with a bladder-type press, centrifugation and ultrafiltration to produce a clarified apple juice extract. This clarified juice was analysed by Blicq (private communication, 1988) for the sensory attribute of "freshness", and found to be similar to two commercial brands of apple juice.

d. "Ultrapress" ultrafiltration technology. Barefoot et al. (1989), and Thomas et al. (1987) recently described a new technology for the production of clarified apple juice. The process system, which has recently been promoted on a commercial basis, consists of:

i) comminuting in a hammer mill;

ii) addition of a pectinase-type enzyme;

iii) simultaneous extraction of juice from pulp combined with ultrafiltration of the juice. This was achieved by pumping the apple

mash in pressurized tangential flow against an ultrafiltration membrane deposited on the inside surface of a sintered stainless steel support tube. The ultrafiltered apple juice extract permeated through the ultrafiltration membrane and then through the pores of the support tube. The pressure used was 2070 kPa at a temperature of 50°C. The retentate was concentrated into a thick slurry after a single pass and did not require recirculation to the feed tank. The process system would need to be evaluated for raspberry before a judgement of its probable utility could be made. Of concern might be the effect of heat on anthocyanin degradation (Mazza and Brouillard, 1987), and on possible losses of volatile aromas.

e. Contemporary clarified raspberry juice technology. One major North American raspberry juice processing company has been reported by S. Lazzari and R. Martens (1986, personal communications) to utilize the following process operations for clarified juice production:

i) crushing partially thawed raspberries;

ii) enzyme treatment (probably pectinases) at 50°C;

iii) juice extraction with a continuous belt press;

iv) further enzyme treatment (probably also pectinases);

v) centrifugation;

vi) final clarification (method not identified).

The use of pectinase enzymes would prevent the formation of pectin gels in the crushed fruit, facilitating juice extraction. Subsequent pectinase treatment would facilitate the precipitation of suspended particles for separation by centrifugation. The use of

elevated temperatures for the enzyme reaction has been reported to typically result in greater juice yield than reaction at lower temperatures (Reed and Underkofler, 1966, Luh et al., 1986, Pederson and Beattie, 1943). Elevated temperatures, however, might be of concern with respect to possible anthocyanin degradation, as well as possible loss of volatile aroma compounds.

III. MATERIALS AND METHODS

A. INVESTIGATION OF RASPBERRY JUICE EXTRACTION

1. MATERIALS

a. Raspberry fruit. Raspberries were harvested in July of 1986 from the biennial fruiting cultivar "Boyne". The perennial rootstock from which the fruit bearing canes had emerged was relatively young, having been planted three or four years previously. The plants were grown on the organic soil of a peat bog in south-eastern Manitoba, in which the peat varied in depth from approximately two to four meters. The peat bog had been partially drained such that free water was available to the roots at approximately one to two meters depth. Foliar application of micronutrients had been used to compensate for the deficiency of native organic soils in some of the micronutrients essential for plant growth. Harvesting was done manually. The raspberries were placed into 11.4 litre plastic pails with lids, with each pail holding approximately nine kilograms. These pails were placed into a freezer unit at approximately -10°C, becoming fully frozen within one day of picking. The pails were then moved to a frozen storage room and stored at approximately -35°C for nine months. When required for experimental work, the pails were thawed for two days at approximately 16°C.

b. Enzymes. The commercial pectinase-type enzyme preparations evaluated were:

i. "Clarex L" (Miles laboratories, Inc., Elkhart, IN). The manufacturer stated that the pectic enzymes in this product hydrolyze and depolymerize pectins and had an activity equal to "15,000 AJDU/mL" (AJDU = apple juice depectinization units). AJDU/mL had been determined by Miles Laboratories procedure no. ME 400.16, which is based upon the time required to depectinize an unclarified apple juice substrate at 3.5 pH and 45°C, with the end point determined by isopropyl alcohol precipitation, and then correlating with a pectinase standard of known activity. The product was said to also contain cellulase, hemicellulase and protease. The manufacturer recommended its addition to raspberry during the crushing operation prior to juice extraction at a rate of 25 to 75 mg/kg of fruit.

ii. "Pectinex 3XL" (Novo Ferment Ltd., Basel, Switzerland). The manufacturer stated that this product contained enzymes of Aspergillus niger origin, consisting mainly of pectintranseliminase, poly-galacturonase, pectinesterase and hemicellulases. The activity was stated to be "3000 FDU 55°/g" (FDU =" ferment depectinization units"), meaning that one gram was capable of depectinizing 100 L of a standard non-clarified pasteurized apple juice at 55°C within two hours, as indicated by a reduction in viscosity. The manufacturer recommended its addition to raspberry during the crushing operation prior to juice extraction at a rate of 100 mg/kg of fruit.

iii. "Pectinex Ultra SP-L" (Novo Ferment Ltd., Basel, Switzerland). The manufacturer stated that this product contained

enzymes of Aspergillus niger origin, similarly consisting mainly of pectin-transeliminase, polygalacturonase, pectinesterase and hemicellulase. The activity was stated to be "26,000 PG (pH 3.5)" (PG = "polygalacturonase units"), relating to the reduction of the viscosity of a solution of pectic acid. The manufacturer recommended its addition to raspberry during the crushing operation prior to juice extraction at a rate of 200 mg/kg of fruit.

2. EXPERIMENTAL OBJECTIVES AND DESIGNS

a. Enzyme, temperature and time treatments.

The objective of the first experiment was to determine the effects and interactions of mixed enzymes addition, time and temperature on crude juice extraction and degradation of macromolecules. A second objective was to evaluate the relative effectiveness of the three above described commercial mixed enzymes products when used at the maximum rates recommended by their manufacturers, in order to obtain at least partial information on which to base a purchase decision for the enzyme product to be used in subsequent pilot scale processing trials. Maximum recommended rates for each product were used in order to avoid the possibility of performance failure by a particular enzyme product being reasonably attributable to inadequacy of rate of addition. No enzymes were added for the control treatment. At the ambient temperature of 18°C, reaction times of 1.5 hours, 4.5 hours and 18.0 hours were tested. These time intervals covered the range of times suggested by the enzyme product manufacturers for various fruit applications. For the 4.5 hour treatment the effect of 50°C was also evaluated, this temperature having

been indicated by the manufacturer as being optimum for the Clarex L product, and also having been used for juice processing by other researchers (Pederson and Beattie, 1943, Thomas et al., 1986). Uniform samples of thawed raspberry of approximately 150 grams in weight were put into 250 mL Erlenmeyer flasks. The enzyme products were added to the flasks in a random order at two minute intervals, at the manufacturers' recommended rates. A no enzymes added treatment served as a control. Mixing was achieved by hand stirring with a spatula for eight seconds; hand stirring was applied to each treatment as uniformly as possible. Constant temperature was maintained with controlled temperature water baths. Each treatment was conducted in triplicate. Upon completion of the exposure time, the treatments were evaluated at two minute intervals, in the same order as they were initiated. Evaluation consisted of analyzing for "crude juice extraction" with a "Bosch" juice extractor (method described in section III-D), and for "volumetric alcohol precipitation" (method described in section III D) to provide an indication of the extent of degradation of pectin and other macromolecules.

b. Maceration, water dilution and enzyme treatments.

The objective of the second experiment was to determine the effects and interactions of maceration, water dilution, and mixed enzymes addition on crude juice extraction and degradation of macromolecules. Three physical treatments were tested on thawed raspberry samples of approximately 150 grams weight. The two "Pectinex" brand enzyme products were also compared, both at the same 200 mg/kg rate in order to

determine whether or not differences in performance noted in the first experiment could be attributed to differences in product volume added. No enzymes were added for the control treatment. All treatments were conducted in triplicate and analysed after one hour enzyme reaction for "crude juice extraction" (method described in section D), and for "volumetric alcohol precipitation" (method described in section D). The three physical treatments were:

i. A "blender" treatment consisted of macerating the thawed raspberry with a "Sunbeam" model "Osterizer" food blender (Consumers Distributing, Winnipeg) operated at high speed for eight seconds, mixing in the added enzyme product at the same time.

ii. A "stirring" treatment consisted of hand stirring the thawed raspberry with a spatula for eight seconds, disrupting most whole berries into constituent intact and broken drupelets, mixing in the added enzyme product at the same time.

iii. A "drip/dilute" treatment consisted of simply allowing a free run crude juice extract (FRCJE) to drip for one hour by gravity through a suspended domestic kitchen sieve of 3 mm wire mesh onto which the thawed raspberry was placed. The partially dejuiced raspberry pulp was then transferred to an Erlenmeyer flask, distilled water added at the rate of 50% of the starting weight of the thawed raspberry, and then stirred manually with a spatula for eight seconds, mixing in the added enzyme product at the same time. For this treatment, the total crude juice extraction (CJE) was calculated as follows:

(CJE) = (FRCJE) + (CJE for enzyme treated diluted pulp) - (added water).

c. Physical pre-treatments, enzyme, pressing and time treatments. The objective of the third experiment was to determine the effects and some interactions of different physical pretreatments, mixed enzymes addition, enzyme reaction time, and the use of a bladder-type fruit press, on "dried centrifugal solids" (method described in section D), "hypothetic clarified juice extraction", and maximum visible light absorption of the juice extract obtained (method described in section III-D). Hypothetic clarified juice extraction (HCJE) is equivalent to the potential clarified juice content of the crude juice extract, based upon the assumption that clarification would require the removal of all dried centrifugal solids, and is calculated as follows:

(HCJE) = (% CJE) - [(% CJE) x (% dried centrifugal solids)]

Five treatments were compared using weighed frozen raspberry samples of 8.0 to 10.0 kg (each sample consisted of the entire contents of one pail of raspberries as packed at harvest), and each treatment was replicated three times. The treatments were:

i. The pails of frozen raspberries were allowed to partially thaw at room temperature, and then the remaining semi-frozen central core was sheared with a knife into 5 cm cubes, placed into an open tray and frequently stirred manually to hasten thawing and warming to 18°C. The thawed raspberry was put back into the pails and Pectinex Ultra SP-L was added at 200 mg/kg, stirred into the thawed raspberry, and then the pails closed and vigorously shaken for 10 seconds to further distribute the enzyme and disrupt raspberry tissue structure. The pails were left undisturbed for 17 hours, at which time it was again vigorously shaken for 10 seconds, and then left undisturbed for a further one hour. The

entire contents of each pail were then separately placed into a Willmes fruit press and the crude juice extracted (method and equipment described in section B.2.b.).

ii. The frozen raspberries were placed in a room at 4°C, removed from the pails and immediately smashed into drupelets. This product was then put back into the pails and the pails placed at room temperature to finish thawing and warm to 18°C. Pectinex Ultra SP-L was added at 200 mg/kg, stirred into the thawed raspberry, and then the pails closed and vigorously shaken for 10 seconds. The pails were left undisturbed for 18 hours. The entire contents of each pail were then separately placed into a Willmes fruit press and the crude juice extracted.

iii. The pails of frozen raspberries were allowed to fully thaw at room temperature. Pectinex Ultra SP-L was added at 200 mg/kg and stirred into the thawed raspberry with a wire wisk which separated most berries into individual druplets, broken druplets and clumps of drupelets. The pails were then closed and vigorously shaken for 10 seconds. The pails were left undisturbed for 1.0 hour, again vigorously shaken for 10 seconds, and then left undisturbed for 0.5 hour. The entire contents of each pail were then placed into a Willmes fruit press and the crude juice extracted.

iv. The pails of frozen raspberries were allowed to completely thaw at room temperature. Pectinex Ultra SP-L was added at 200 mg/kg, the pails closed and then gently inverted twelve times to distribute the enzymes with minimal disruption of berries. The pails were left undisturbed for 1.0 hour, again gently inverted twelve times, and then

left undisturbed for 0.5 hour. The entire contents of each pail were then placed into a Willmes fruit press and the crude juice extracted.

v. The frozen raspberries were removed from each pail and placed into stainless steel wire strainers of 6.0 mm mesh to thaw at 4°C. As they thawed, a free run crude juice extract passed through the strainers and dripped into clean pails below. The strainers retained the solid raspberry tissues, which was not further treated.

B. CLARIFIED RASPBERRY JUICE PROCESSING TRIAL

1. MATERIALS

a. Raspberry fruit. Raspberries used for the raspberry juice production run of July 29, 1987 were of the same origin as used for the above investigation of juice extraction. Raspberries processed on February 4, 1988 were also of the same origin, but were harvested the following year in July of 1987 and compacted into 11.4 litre plastic pails with lids, with each pail holding approximately 10 kilograms. An unknown portion of the berry drupelets were ruptured by the compaction such that approximately half of the raspberries were submerged in juice. The pails were placed into a freezer unit at approximately -10°C, becoming fully frozen within one to three days of picking, depending upon the frequency of opening the freezer unit door and the quantity and stacking arrangement of the pails of field temperature raspberries put into it each day. The pails were then moved to a commercial frozen storage warehouse and stored at approximately -20°C for twelve months. When required for experimental work, the pails were thawed for two days at approximately 16°C. Raspberries processed June 16, 1988, July 14, 1988 and September 29, 1988 were mixtures of 1987 peat soil grown raspberries, with 1987 raspberries which had been grown on mineral soils in south-central Manitoba. The January 13, 1989 processing run used entirely 1987 mineral soil grown raspberries.

b. Enzyme. "Pectinex Ultra SP-L" (Novo Ferment Ltd., Basel, Switzerland) (described above), was used.

2. PROCESSING TRIAL

Refer to process operations flow diagram (Figure 6).

a. Enzyme addition and reaction. The commercial enzyme Pectinex Ultra SP-L was mixed by aggressive manual stirring into the thawed raspberry at the manufacturer's recommended rate of 200 ml per tonne of raspberry. The enzyme treatment was for 16 to 22 hours as the raspberry mash warmed to the ambient room temperature of 16°C.

b. Pressing. Following enzyme treatment, the raspberry mash was placed into a pilot scale "Josef Willmes" "Model Willmes Presser type 60" (Josef Willmes Gmbh, Bensheim, W. Germany) cylindrical bladder fruit press, in lots of approximately 5 kilogram size. The pressure within the bladder, squeezing the raspberry mash against the press cloth, was set at approximately 5 bars. The press cloth held back the seeds and the insoluble large debris of the press cake, allowing a crude juice extract to permeate through the press cloth and be collected in a stainless steel tray below. The press cake was then removed from the fruit press and the process repeated. The crude juice extract was put into 20 litre plastic pails with lids and stored at 4°C for 16 hours.

c. Centrifugation. To remove insoluble plant material which would plug the hollow fibres in the ultrafiltration step (described below), the crude juice extract obtained by pressing was continuously fed with a positive displacement gear pump into a "Westfalia Separator" "Model SB-7-06-076" desludger centrifuge (Centrico, Inc., Northvale, NJ). The

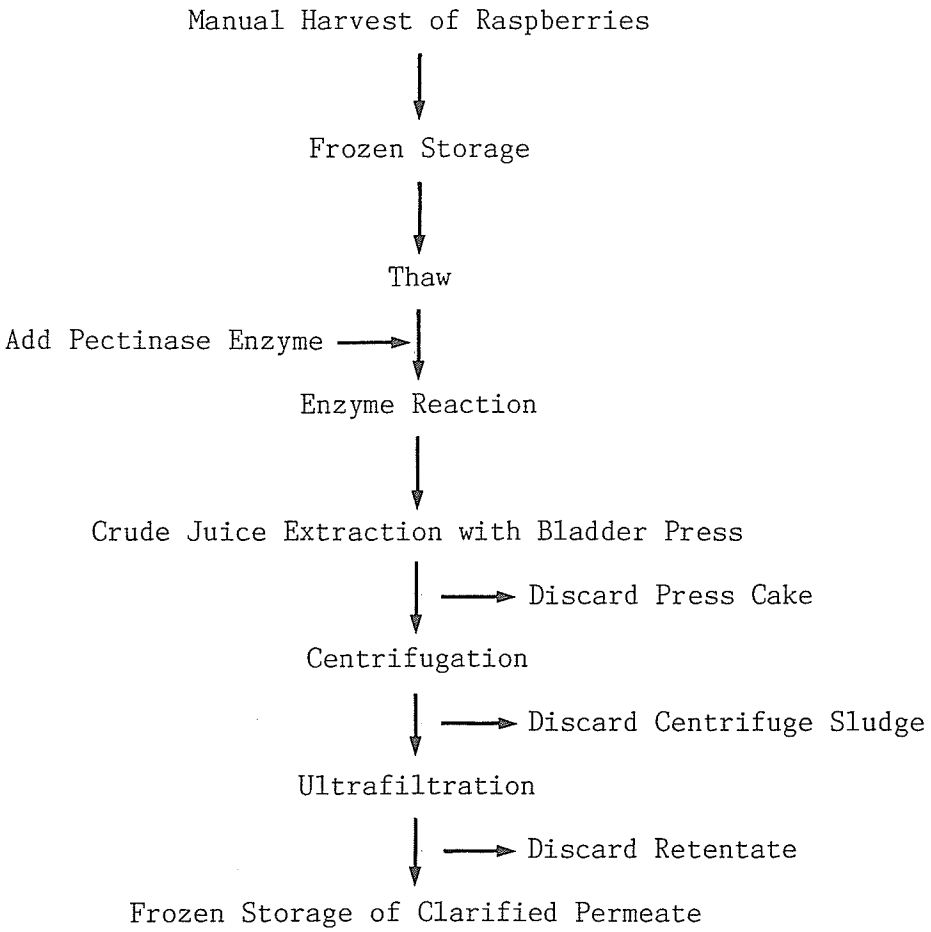


FIGURE 6. FLOW DIAGRAM OF RASPBERRY JUICE EXTRACTION AND CLARIFICATION.

manufacturer rated the clarifier as developing a relative centrifugal force equivalent to approximately 10,000 to 11,000 x g (gravitation force). The greater density of the insoluble sludge fraction held it at the circumference of greatest radius inside the rotating bowl, while the lighter density juice extract fraction flowed back to the centre of the bowl and exited from the top (Figure 7). Under continuous feed, the exposure time of the juice extract, between entering and exiting the bowl, was approximately 30 seconds. As required, the continuously rotating bowl was allowed to briefly open at its point of greatest radius in order to permit the accumulated sludge to exit. The sludge so obtained was combined and rerun through the centrifuge to further concentrate it and recover more of the juice extract. The centrifuge product was put into 20 litre plastic pails with lids and stored at 4°C for 16 hours.

d. Ultrafiltration

i. Ultrafiltration processing. Following the removal of most insoluble solids by centrifugation, the juice extract (centrifuge product) was ultrafiltered. This was accomplished with a "Romicon" "Model HF 26.5-43 PM50" hollow fibre cartridge (Romicon Inc., Woburn, MA), operated on a "Romicon""Model HF2S" pilot plant system (Romicon Inc., Woburn, MA). The manufacturer rated the polysulphone membrane formed on the inside surface of the porous hollow fibres to have nominally 50,000 molecular weight cut off (MWCO) separation properties, meaning that molecules and other substances larger than approximately 50,000 molecular weight would be retained inside the hollow fibre, and

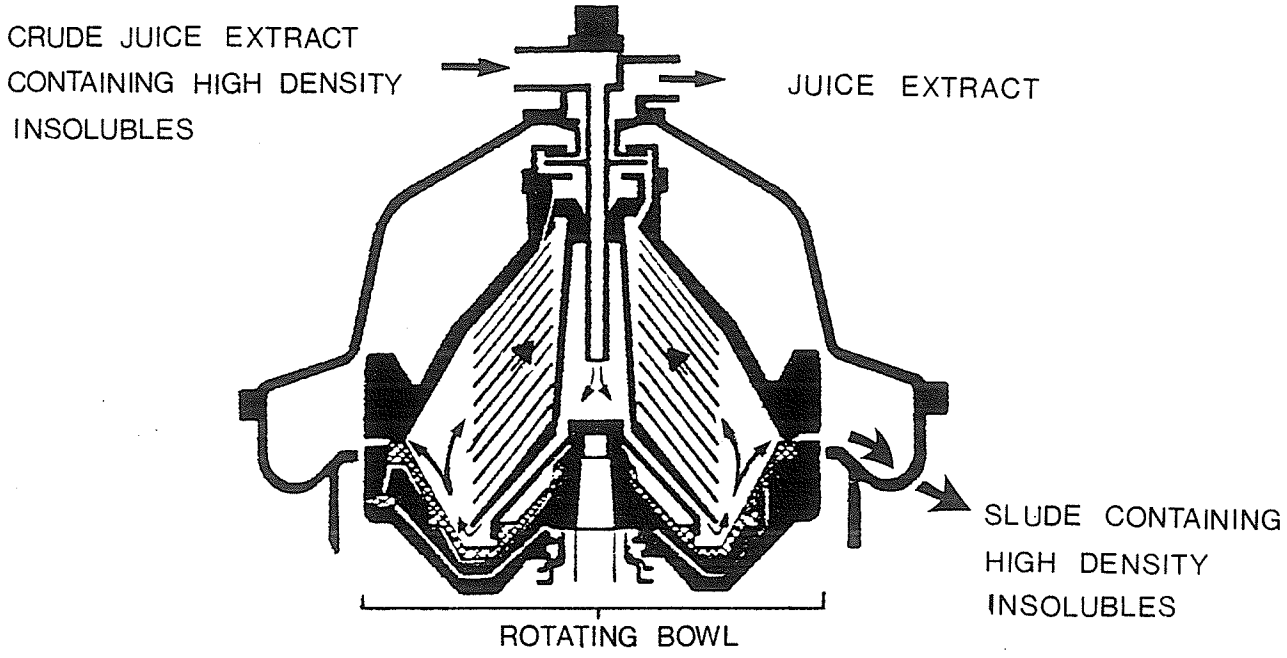
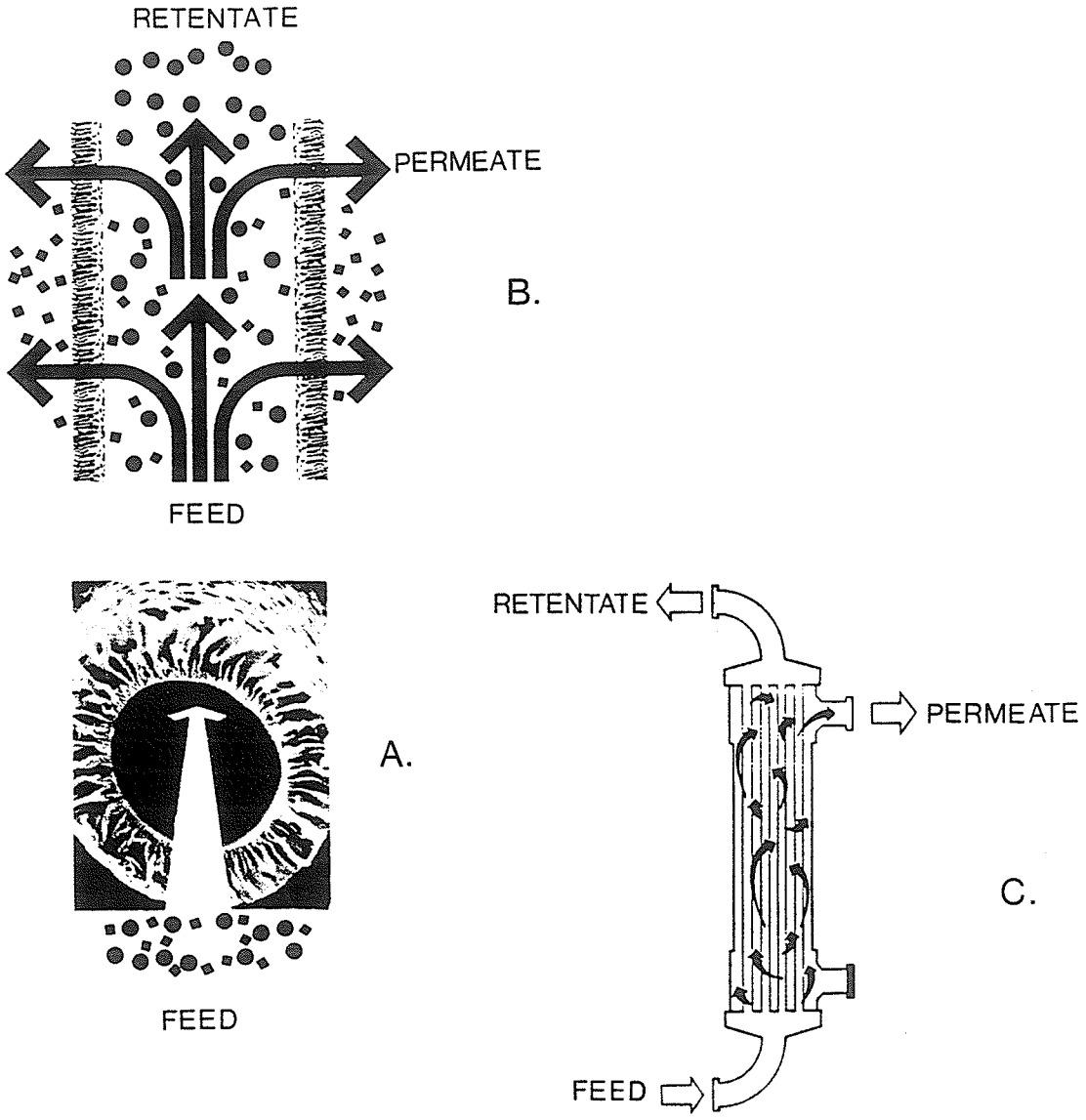


FIGURE 7. SCHEMATIC DIAGRAM OF A CONTINUOUS DESLUDGER CENTRIFUGE. FROM WESTFALIA SEPARATOR.

smaller molecules dissolved in the juice extract would be expected to permeate through the membrane and through to the outside of the hollow fibre. The hollow fibres were contained within a cylindrical tube which served to collect the "permeate" fraction. The juice extract retained inside the hollow fibres (the "retentate") was continually returned to the feed tank to be mixed and recirculated through the hollow fibre cartridge, achieving a tangential flow membrane filtration process (Figure 8). For normal operation, both the valve regulating the feed entry to the cartridge and the valve regulating the retentate exit from the cartridge were left fully open such that there was zero retentate backpressure at the exit from the cartridge.

ii. Observation of the effect of retentate backpressure on permeate flux. The effect of changing the retentate backpressure on the permeate flux for water was compared with its effect on the permeate flux for raspberry juice extract. Retentate backpressure was changed by adjusting the valve regulating it's exit from the cartridge. The first trial with water was at 18.3°C and was conducted immediately prior to the trial with raspberry juice extract at 5.3°C. The second trial with water was at 3.9°C and was conducted at a later date using the same equipment, although the centrifugal pump appeared to be of lower performance and the ultrafiltration membrane permeability characteristics may have altered. These trials were not replicated because of the increased difficulty in cleaning the membrane following each trial at increased retentate backpressure, suggesting that membrane fouling resulted from the trials.



Key:

● = High molecular weight solute.

▪ = Low molecular weight solute.

FIGURE 8. DIAGRAM OF HOLLOW FIBRE ULTRAFILTRATION.
A: PHOTOMICROGRAPH OF A CROSS SECTION OF A HOLLOW FIBRE WITH AN ULTRAFILTRATION MEMBRANE ON THE INSIDE SURFACE (approx 25x).
B: SCHEMATIC LONGITUDINAL SECTION OF A HOLLOW FIBRE SHOWING PATH OF SOLVENT AND SOLUTES. C: SCHEMATIC LONGITUDINAL SECTION OF A CARTRIDGE HOLDING MANY HOLLOW FIBRES. FROM CHERYAN (1986), AND BRESLAU (1984).

e. Product storage. Following ultrafiltration, the permeate fraction was collected in 11.6 litre plastic pails with lids, frozen and stored at approximately -20°C for one week to three months and then supplied to the University of Manitoba Food Science Department Dairy for use in a juice beverage.

C. INVESTIGATION OF SUCROSE INVERSION IN RASPBERRY JUICE

1. MATERIALS

a. Raspberry fruit. Investigations were conducted using Boyne cultivar raspberries of different origins and storage treatments as follows:

i. Investigations conducted in February 1988 used raspberries which were manually harvested in 1986 and 1987 from plants grown on a peat bog in south-eastern Manitoba, and stored frozen (as described in sections A.1.a. and B.1.a above). Compaction under their own weight, and drupelet rupture due to water crystallization and expansion during freezing, resulted in the raspberries being submerged in their own juice upon thawing.

ii. Investigations conducted in July to August of 1990 used both frozen stored and fresh raspberries grown on a mineral soil in southern Manitoba. The frozen stored raspberries were manually harvested in 1988 and compacted into 11.4 litre plastic pails with lids, with each pail holding approximately 10 kilograms. Many of the berry drupelets were ruptured by the compaction such that approximately half of the raspberries were submerged in juice. The pails were placed into a freezer at approximately -10°C, becoming fully frozen within one day of picking, and then transferred one week later to a freezer at approximately -20°C and stored for two years. The raspberries were found to be submerged in their own juice upon thawing.

The fresh raspberries were manually harvested in 1990. Within one hour of harvesting, the fresh raspberries were placed into an insulated picnic cooler with a frozen ice-pack in order to commence cooling, and

then within four hours placed at approximately 4°C and stored for seven to 64 hours.

b. Reagents. Citric acid (anhydrous) "A940-1", lot CL 7044197, supplied by "Fisher Scientific" (Nepean, Ontario), was used for the acidification treatment. "Pectinex Ultra SP-L" pectinase enzyme product manufactured by "Novo Ferment" (as described in section A.1.b. above) was used for the pectinase enzyme addition treatment.

2. EXPERIMENTAL OBJECTIVES AND DESIGNS

a. Preliminary observations. HPLC analyses for sugars (method described in section D. below) were conducted on several raspberry juice extract treatments. Each analysis required thirty minutes of HPLC operating time, which necessarily limited treatment replications for some of the preliminary observations. The preliminary treatments were:

i. A pectinase-treated, pressed, centrifuged and ultrafiltered raspberry juice extract processed from frozen 1987 peat soil grown raspberries (as described in section B. above) was analysed in triplicate in February 1988.

ii. A free-run juice extract from partially thawed frozen 1986 peat soil grown raspberries was also analysed in triplicate in February, 1988. These were analysed within 1.0 hour of thawing at 4°C.; the samples warmed to the ambient room temperature of approximately 23°C during this hour.

iii-a. Two samples of free-run juice extracts from partially thawed frozen 1988 mineral soil grown raspberries were analysed in July 1990. Both treatments were analysed within 0.2 hour of removal from

4°C; the samples warmed to 23°C during the necessary preparation steps prior to HPLC injection. This sought to determine if the absence of sucrose in the raspberries analysed earlier was possibly related to the peat soil in which they had been grown, or rather possibly attributable to handling or storage factors.

iii-b. Two samples of the same origin as iii-a were similarly analysed immediately after the addition of pectinase enzyme, to determine if this had an effect.

iv. Juice extracts from samples of twenty 1990 mineral soil grown raspberries were analysed following storage at 4°C for 36 hours:

iv-a. One treatment consisted of crushing the raspberries at 23°C, and then analysing after 0.2, 1.5, 4.5, 7.5 and 10.5 hours at 23°C.

iv-b. A second treatment consisted of subjecting the whole raspberries to pasteurization at a temperature of 80°C for 30 seconds during crushing and then analysing after 0.2, 1.0 and 7.5 hours at 23°C. This sought to determine whether or not sucrose inversion was due to biological metabolism, eg. plant or microbial invertase enzyme activity, these possibly being inactivated by a pasteurization treatment.

iv-c. A third treatment also used 80°C for 30 seconds during crushing, followed by acidifying with the addition of approximately 2.5% (wt/wt) citric acid as required to lower the pH from 3.1 to 2.6. This was also analysed after 0.2, 1.0 and 7.5 hours at 23°C. This sought to determine whether or not acid hydrolysis of sucrose was a significant factor in the observed sucrose inversion, this treatment being expected to result in accelerated inversion if such was the case.

v-a. to v-f. Six samples of twenty-six 1990 mineral soil grown raspberries were crushed and subjected to different temperature treatments following storage at 4°C for 21 hours. One sample was analyzed within 0.2 hour of crushing at 23°C. The other five samples were analyzed following crushing and holding for 1.0 hour at 23°C, 45°C, 55°C, 65°C and 75°C respectively. This had the objective of determining temperatures for possible enzyme activation and denaturation. Unfortunately, this experiment could not replicated due to HPLC equipment failure.

vi-a. Free-run juice extracts from triplicate samples of twenty frozen whole 1990 mineral soil grown raspberries were analysed. Within seven hours of harvesting and cooling at 4°C, these raspberries were put into 250 mL Erlenmeyer flasks and placed at approximately -10°C to freeze. After 24 hours the flasks were moved to 4°C and the raspberries allowed to thaw. Samples were taken of the juice which had leaked from the whole raspberries and collected in the bottom of the flasks after ten hours of thawing. The samples were analysed after 0.2 hour, 0.5 hour and 1.0 hour at 23°C. This sought to determine if sucrose inversion was a simple function of freezing and thawing.

vi-b. Free-run juice extracts from triplicate samples of the same origin and freeze-thaw treatment as vi-a were analysed after addition of pectinase enzyme and reaction for 0.5 hour, 1.0 hour and 1.6 hours at 23°C. This sought to determine if invertase activity was present in the commercial pectinase enzyme product.

vii. One sample of twenty immature 1990 mineral soil grown raspberries, in the firm yellow-white stage of development showing the

beginning of orange-pink pigmentation, was analysed after storage at 4°C for 36 hours, and within 0.2 hour of crushing. This served to provide an indication of the relative levels of the three sugars prior to ripening.

b. Investigation of time, pasteurization and acidification treatments.

1. The treatments conducted in section (a.iv.) above were replicated in triplicate using fresh whole raspberries which had been stored at 4°C in order to validate the initial observations made. Following crushing, the three treatments were held at 23°C and analysed after 0.2, 1.5, 3.0 and 4.5 hours. The first replication used 1990 mineral soil grown raspberries which had been stored for 14 hours at 4°C. Raspberries which had been stored for 38 hours at 4°C were used for a second replication, and raspberries which had been stored for 64 hours at 4°C were used for a third replication. These different storage times for each replicate were necessitated by the long time required for HPLC analysis and the long distance between the raspberry field and the HPLC laboratory. The use of different storage times, however, allowed for the simultaneous evaluation of whether or not different storage times would influence the response to the three treatments applied. It was shown that different storage times did not affect the response of invert sugar level to the treatments of crushing, crushing after pasteurization and crushing after pasteurization and acidification.

ii. Neither the crushing after pasteurization treatment, nor the crushing after pasteurization and acidification treatment, significantly

altered the initial level of invert sugar at 0.2 hours compared to that obtained for simply crushing for each of the above three replications nor for the earlier replication described in section (a.iv.). These three treatments could therefore be regarded as equivalent replicates at 0.2 hour analysis for each storage time at 4°C. These values were therefore suitable to establish the effect of storage time at 4°C on the level of invert sugar as a percentage of total sugars in whole raspberry.

D. ANALYTICAL METHODS

a. Crude juice extraction. A standard laboratory method was developed to evaluate the relative ease of crude juice extraction from thawed raspberry when subjected to various pretreatments. A "Bosch" model "UM3" (Bosch, Germany) universal food mixer/juice extractor was utilized for this purpose. The juice extractor consisted of a spinning bowl with a solid bottom and perforated walls. The wall perforations were approximately 2.0 mm in diameter. Approximately 200 grams of treated raspberry were fed into the centre of the spinning bowl from where it was immediately flung against the inside of the bowl wall and held there by centrifugal force. The insoluble pulp remained inside the bowl as the crude juice was separated by passing through the perforations into a collection chamber outside of the bowl. After ten seconds the juice extractor was turned off. The crude extracted juice was then weighed and expressed as a percentage of the feed.

b. Volumetric alcohol precipitation. A rapid analytical method for assessing the extent of enzymatic degradation of macromolecules was adapted from D'Souza (1986) and Novo Ferment (1986). A 5.0 mL aliquot of raspberry juice extract was added to a test tube containing 10.0 mL of 96% ethyl alcohol. After 15 minutes a visual assessment was made of the proportion of the total liquid volume in which flocculation occured as a result of the interaction of the juice extract with the ethyl alcohol; for example, a rating of 0.60 was assessed if floc was present in 60% of the total liquid volume. Flocculation is indicative of the

presence of non-hydrolysed pectin (Novo Ferment, 1986) and possibly of other colloidal polysaccharides and proteins as well (Joslyn, 1970).

c. Total solids. A.O.A.C. (1984) method 22.018 was used with the following modifications: Samples of 11.0 to 12.0 grams weight were dried in metal weighing dishes for 16 hours in a forced air oven at 70°C. Earlier trials by a co-worker using a vacuum oven for drying raspberry products indicated difficulty in controlling excessive foaming and dish overflow during the drying process. A forced air oven was selected instead to ensure consistency of results in this study.

d. Centrifugal solids. A laboratory assessment of the efficiencies of the desludger centrifuge and of the ultrafilter in removing juice extract fractions of high specific density was developed. Samples of approximately 9.0 to 11.0 grams weight were centrifuged in a "Sorval" model "GLC-1" centrifuge for 20 minutes at a relative centrifugal force equivalent to approximately 2,200 x g. The supernatant was then decanted and the precipitate pellet weighed and expressed as a percentage of the sample. This analytical treatment differed from the centrifugation process operation using the "Westfalia Separator" continuous feed, intermitten desludging centrifuge (described above). This laboratory assessment used a much slower rate of product acceleration to maximum centrifugal force, a much lower maximum relative centrifugal force, and a much longer time of exposure to the maximum centrifugal force.

e. Dried centrifugal solids. Samples of 28.0 to 30.0 grams weight were analysed for centrifugal solids (as described above), with the precipitate pellet obtained subsequently analysed for total solids (as described above). This dried pellet was weighed and expressed as a percentage of the original sample.

f. Soluble solids. A.O.A.C. (1984) method 31.011 was followed using a "Carl Zeiss" "Model 47705" refractometer (Carl Zeiss, Germany) with direct reading in percent sucrose. Because the HPLC analyses of sugars (described below) showed primarily fructose and glucose to be present, the direct readings from the sucrose scale were increased by the amount of 0.022 for each 1.0% reading, as suggested in method 31.011 for liquid products containing invert sugar, and as discussed by Joslyn (1970).

g. Titratable acidity. A.O.A.C. (1984) method 22.059 was followed, except for the glass electrode pH meter being standardized to 4.00 pH to facilitate accurate measurement of the initial pH of the unadulterated juice extract concurrent with measurement of total acidity by titrating to pH 8.1. This reduced the possibility of measurement errors which may have resulted from instrument recalibration to two different pH levels for standardization, and also minimized the chance of sample alteration from microbial fermentations during the time lag required for recalibration. Instrumental pH readings differed by approximately 0.02 pH depending upon whether or not it was standardized to pH 4.00 or pH 7.00.

h. High performance liquid chromatography of sugars. For the identification and quantification of major sugars in raspberry juice extracts, an HPLC method similar to that described by Sharma et al. (1988) was followed. A 300 mm x 7.8 mm "Aminex HPX-87P" carbohydrate analysis column supplied by "Bio-Rad Laboratories" (Richmond CA) was used for the analysis. The column was placed inside a temperature controlled oven and connected to a "Waters" HPLC system (Waters Associates, Milford, MA) consisting of a Model "M6000 A" solvent delivery system, Model "U6K" sample injector and a model "R 401" differential refractive index detector. Deaerated distilled water was used as the eluent. The flow rate was 0.60 mL/min. During analysis the column temperature was maintained at 85°C. Attenuation of the refractive index detector was set at 32X. Recording and analysis of the detector response were conducted on a "Vista 401" data system (Varian Instrument Group, Walnut Creek, CA). Purified sucrose, alpha-D-glucose, beta-D-glucose, D-galactose and D-fructose manufactured by "Alltech Associates Inc." and purchased from "Sigma Chemical Co." (St. Louis, MO), were used as standard sugars. These sugars were dissolved in water, at known concentrations in the range of 3.40 to 4.20 mg/mL, and filtered through a "Waters""Sep-Pak" 0.45 micron silica filter cartridge (Waters Associates, Milford, MA) to remove particulate impurities prior to injection of a 20.0 microlitre aliquot onto the HPLC column. The standards were run three times and the average values for retention times and peak areas used as standard references. Clarified raspberry juice extract samples for analysis were thawed and filtered through a

"Waters" "Sep-Pak" C-18 cartridge (Waters Associates, Milford, MA) to remove compounds of low polarity, and then through a "Waters" "Sep-Pak" 0.45 micron silica filter cartridge (Waters Associates, 1981). For the February 1988 analyses, sample aliquots of 5.0 microlitres were injected onto the column; for the July-August 1990 analyses, samples were diluted to 25% concentration prior to injections of aliquots of 20.0 microlitres. Sugars present in the sample were identified by comparison of the retention time of each peak with those of the standard sugars. The concentration of each sugar in the sample was calculated by comparison of the area of its peak to the area of the calibrated sugar of known concentration (Figure 13 and 14 in appendix).

i. Light absorption by scanning spectrophotometer. A "Hewlett Packard" model "8451 A" diode array spectrophotometer (Hewlett Packard Canada Ltd., Mississauga, Ontario) was used to measure the light absorption characteristics of the ultrafiltration retentate and permeate over the range of wavelengths from 380 nm to 820 nm. The raspberry juice extract samples were held at 4°C in the dark for 20 to 44 hours prior to analysis. The pH for all samples analysed was unadulterated at 3.0. The samples were diluted 1:20 with distilled water for analysis.

j. Microbiological analyses. Microbiological analyses were conducted on ultrafilter feed and permeate samples within two hours of the ultrafiltration process operation. Method no. "MSHPB-18" of the "Health Protection Branch" (Department of Health and Welfare, Government of Canada) was followed for standard plate counts (SPC). Yeasts and

moulds were enumerated by the acidified method as described by Speck (1984). These analyses were conducted by the Microbiology Department of the "Canadian Food Products Development Centre" at Portage la Prairie, Manitoba, operated by the Manitoba Research Council.

k. Sensory analysis of a formulated raspberry juice beverage. A juice beverage was formulated from the ultrafiltered Boyne raspberry juice extract with the addition of distilled water, sucrose and citric acid. This formulated product was evaluated for preference against two commercial raspberry juice beverages currently retailed under the "McCain" and "Townhouse" labels. The preference evaluation was performed using controlled sensory analysis techniques as described by Larmond (1977). The panelists were passers-by in a major Winnipeg shopping centre who were presented with the three raspberry juice beverages in random order in clear plastic glasses, identified only by non-descriptive three digit numbers. The panelists were asked to rank the three products on a nine point hedonic scale indicating their degree of like or dislike for each product, with "-4" corresponding to "dislike extremely" and "+4" corresponding to "like extremely" . (A copy of the sensory analysis ballet is in the appendix.) The panelists were also asked to indicate their age category and the cultural background in which they were raised. Only the preference evaluations of persons who were raised on the Canadian Prairies were retained for data analysis, as these persons represented the probable target market for the product. The evaluations of persons under the age of eighteen years were excluded due to the relatively small number of these persons in the sample.

Statistical analyses of variance of the data were tested using Tukey's test.

1. Statistical analyses of data. Standard deviations were calculated for means, and regression was used to estimate linearity (Huntsberger and Billingsley, 1977). Two-way and factorial analyses of variance (ANOVA) of the data were tested using Duncan's multiple range test at a probability level of alpha = 0.050 (Snedecor and Cochran, 1980). The analyses of data were conducted using "MSTAT-C" statistical analysis program for microcomputers (MSTAT Development Team, Michigan State University, East Lansing, Mich.; original version (1983) by O. Nissen, Agricultural University of Norway).

IV. RESULTS AND DISCUSSION

A. INVESTIGATION OF RASPBERRY JUICE EXTRACTION

1. EFFECT OF ENZYME, TEMPERATURE AND TIME

Various pectinase, temperature and time treatments were evaluated with repect to their effect upon crude juice extraction from thawed whole raspberries, and with respect to the presence of non-degraded macromolecules (especially pectin) in the extracted juice (Table 4). For the no enzyme added control treatment, crude juice extraction decreased with treatment time, suggesting a gradual formation of stable pectin gels. Pectin gels would decrease viscosity and bind water, solutes and other colloidal substances and insoluble particles (Coultate, 1989). It is likely that some microbial growth will have also occured at 18°C, possibly modifying colloidal composition and viscosity. Volumetric alcohol precipitation (indicative of the presence of macromolecules) of the crude juice extracts also decreased with increased treatment time. Compounds which were present in the extracted crude juice and participated in alcohol induced precipitation for the 1.5 hour treatment, may have instead been retained in the pulp fraction for the longer time treatments as a consequence of the formation of pectin gels. Increasing the temperature to 50°C improved crude juice extraction, which agrees with the findings of Pederson and Beattie (1943). Heating may have increased the solubility of compounds and the

TABLE 4. EFFECT OF ENZYME ADDITION, TEMPERATURE AND TIME ON CRUDE JUICE EXTRACTION FROM THAWED RASPBERRIES, AND ON VOLUMETRIC ALCOHOL PRECIPITATION OF THE CRUDE JUICE.

Enzyme* Product	Temp (°C)	Time (hours)	Crude Juice Extraction (%)	Vol. Alcohol Precip. (%)
No enzyme	18	1.5	50.8 de**	73 b**
No enzyme	18	4.5	41.5 g	50 de
No enzyme	18	18.0	36.1 h	57 cd
No enzyme	50	4.5	47.9 ef	90 a
Clarex L	18	1.5	50.1 de	77 ab
Clarex L	18	4.5	43.7 fg	67 bc
Clarex L	18	18.0	46.7 ef	47 de
Clarex L	50	4.5	60.8 ab	80 ab
Pectinex 3XL	18	1.5	64.7 a	77 ab
Pectinex 3XL	18	4.5	56.8 bc	50 de
Pectinex 3XL	18	18.0	53.3 cd	20 g
Pectinex 3XL	50	4.5	61.0 ab	37 ef
Pectinex Ultra SP-L	18	1.5	60.6 ab	43 def
Pectinex Ultra SP-L	18	4.5	57.7 b	30 fg
Pectinex Ultra SP-L	18	18.0	58.0 b	5 h
Pectinex Ultra SP-L	50	4.5	64.9 a	47 de

Notes:

* Enzyme products added at manufacturers' recommended rates.

** Column values for means followed by the same letter are not significantly different ($P > 0.05$)($n = 3$).

activity of native pectic enzymes (Reed and Underkofler, 1966), reducing the overall viscosity and thereby allowing increased juice release. Microorganisms capable of growth at 50°C would be expected to differ from those which could grow at 18°C, with different effects on viscosity (Ayres et al. 1980).

Addition of the Clarex L enzyme product at the manufacturer's maximum recommended rate was of little benefit with respect to crude juice extraction at 18°C, but was of benefit at 50°C, which is in agreement with the manufacturer's recommendation. Clarex L was of no benefit with respect to volumetric alcohol precipitation remaining in the extracted juice at both 18°C and 50°C, which differs from the usual expectation for pectinase-type enzymes (Coultate, 1989). The reason for this is uncertain.

The two Pectinex products, however, when added at the manufacturer's respective recommended rates, did significantly improve crude juice extraction for all temperature and time treatments relative to the no enzyme added controls, showing no apparent benefit for longer time or higher temperature conditions. Both reduced volumetric alcohol precipitation for the 18°C/18.0 hour and the 50°C/4.5 hour treatments, with the best result being attained with Ultra SP-L at 18°C for 18.0 hours. The Ultra SP-L product also significantly reduced volumetric alcohol precipitation for the low temperature-short time treatments, and thus would be the preferred product on the basis of performance at the rates used.

2. EFFECT OF MACERATION, WATER DILUTION AND TIME

Highest crude juice extraction was achieved with the two stage drip/dilute process involving no physical maceration (Table 5). This process consisting of first collecting the free-run juice upon thawing, and then adding distilled water and mechanically extracting a diluted juice, and then recombining this with the free-run juice. The drip/dilute treatment, however, resulted in a diluted juice extract which might be difficult to market. The maceration treatment with the blender may have caused a greater release into solution and greater mixing of normally cell wall bound pectic substances than the stirring treatment. These pectic substances would have rapidly reacted with water and each other, possibly in conjunction with other released solutes, to form gel matrices increasing viscosity and reducing crude juice extraction (Coultate 1989). For this experiment, the two Pectinex products were tested using the same rates of addition (wt/wt). Both Pectinex enzyme products improved crude juice extraction for all physical treatments, as well as reducing volumetric alcohol flocculation of the crude juice extract, with the only significant difference being the superior performance of Ultra SP-L in the drip/dilute treatment.

3. EFFECT OF PHYSICAL PRETREATMENT, TIME AND PRESSING

The pilot scale juice extraction experiment determined that a greater hypothetic clarified juice extraction was attained when the rate of thawing was first hastened by physical pretreatment and the mixed pectinase enzymes product was added early, allowing reaction to occur for a longer time prior to pressing (Table 6). The early addition of

TABLE 5. EFFECT OF PHYSICAL MACERATION, WATER DILUTION AND ENZYME ADDITION ON CRUDE JUICE EXTRACTION FROM THAWED RASPBERRIES, AND ON VOLUMETRIC ALCOHOL PRECIPITATION OF THE CRUDE JUICE.

Mixing Method	Enzyme* Product	Crude Juice Extraction (%)	Vol. Alcohol Precip. (%)
Blender	Pectinex 3XL	37.1 d**	33 c**
Blender	Pectinex Ultra SP-L	36.5 d	27 c
Blender	No enzyme	19.4 e	70 a
Stirring	Pectinex 3XL	46.4 c	28 c
Stirring	Pectinex Ultra SP-L	51.4 c	28 c
Stirring	No enzyme	32.2 d	73 a
Drip/dilute	Pectinex 3XL	57.2 b	32 c
Drip/dilute	Pectinex Ultra SP-L	63.5 a	23 c
Drip/dilute	No enzyme	49.7 c	45 b

Notes:

* Enzyme products added at 200 mg/kg fruit, at 18°C.

** Column values for means followed by the same letter are not significantly different ($P > 0.05$)($n = 3$).

TABLE 6. EFFECT OF PHYSICAL PRETREATMENT, ENZYME ADDITION, TIME AND PRESSING ON DRIED CENTRIFUGAL SOLIDS IN THE CRUDE JUICE EXTRACT, HYPOTHETIC CLARIFIED JUICE EXTRACTION, AND MAXIMUM VISIBLE LIGHT ABSORPTION.

Treatment	Dried Centrifugal Solids (%)	Hypothetic Clarified % Juice Extraction	Light Absorption at 514 nm
i. Partial thaw, shear, 18 hours enzyme, 18°C, two agitations, press	0.116 b*	84.2 a*	1.03 a*
ii. Frozen break, 18 hours enzyme, 18°C, one agitation, press	0.122 ab	86.2 a	0.90 a
iii. Fully thaw, stir, 1.5 hours enzyme, 18°C, two agitations, press	0.151 ab	79.5 b	
iv. Fully thaw, 1.5 hours enzyme, 18°C gentle mixing, press	0.212 ab	78.2 b	0.97 a
v. Free-run drip, 4°C, no enzyme, no mixing, no pressing	0.222 a	53.6 c	1.01 a

Notes:

* Column values for means followed by the same letter are not significantly different ($P > 0.05$)($n = 3$).

pectinase would have prevented the initial formation of pectin gels, which otherwise would need to be later broken down. Juice extraction was greater for all of the enzyme added and pressed treatments than for the simple one step free-run drip treatment at 4°C using no enzyme, no physical mixing and no pressing. Dried centrifugal solids appeared to be generally higher in the juice extract obtained by the no enzyme free-run drip treatment, possibly due to the lack of added pectinase and hemicellulase enzyme activity which would have otherwise solubilized macromolecules, such as hydrolyzing pectin into constituent sub-units of galacturonic acid and neutral sugars (Coultate, 1989). Maximum visible light absorption of the extracted juice did not significantly differ for the four treatments analysed, suggesting that the pectinase reaction plus pressing combined operation did not alter juice quality with respect to total expressed anthocyanin pigmentation.

B. CLARIFIED RASPBERRY JUICE PROCESSING TRIAL

1. PRODUCT YIELDS

The production run of July 12-14, 1988, which was similar to other production runs with respect to product yields, attained 66.6% recovery of the initial whole raspberry feed in the form of an ultrafiltration clarified juice extract (Table 7). Larger production runs would not suffer equivalent high losses in the minimum hold-up volumes of the centrifuge and particularly in the ultrafiltration equipment. Overall losses with ultrafiltration might be as low as 1.0% to 2.0% of the initial feed for large commercial scale operations (Cheryan, 1986), which would consist of moisture volatization losses, together with the discarded retentate when the minimum equipment hold-up volume for batch processing is reached. Moisture volatization loss may also occur with the pressing and centrifugation operations. The 74.0% recovery rate indicated here as possible for larger scale processing should be compared with the theoretical value of approximately 96% for the maximum recoverable juice extract, achievable only if it was technically possible to remove just the pure insoluble solids fraction, the rest of the whole raspberry being comprised of water and solubles (Table 1).

2. TOTAL SOLIDS AND CENTRIFUGAL SOLIDS

Solids were removed from the crude raspberry juice extract during the centrifugation and ultrafiltration process operations. Although a statistically significant difference was not observed between the total solids content of the raspberry juice extract before and after

TABLE 7. PRODUCT YIELDS DURING RASPBERRY JUICE PROCESSING*.

Sample	Weight (kg)	Proportion of Feed (%)	Adjusted Proportion (%)
Mash to bladder press	628.20	100.0	100.0
Press cake and losses	119.97	19.1	19.1
Crude juice to centrifuge	508.23	80.9	80.9
Sludge and other losses	29.40	4.7	4.7
Hold-up in centrifuge	2.41	0.4	0.1**
Centrifuge product to UF	476.42	75.8	76.1
Hold-up in UF (retentate)	45.00	7.2	2.0***
Other losses in UF	12.92	2.1	0.1**
Ultrafilter permeate	418.50	66.6	74.0

Notes

* Data from the single production run of July 12-14, 1988.

** These losses would be negligible for larger scale processing.

*** Predicted for large scale processing (Cheryan, 1986).

centrifugation and ultrafiltration, the mean values, 7.85 and 7.77 respectively, suggest the possibility of such a trend (Table 8). Accurate sampling of the centrifuge feed was difficult due to the insoluble components tending to settle rapidly, and thus the total solids in the feed may have been under-estimated. Total solids values for the sludge fractions should also be viewed with suspicion because they do not account for unmeasurable moisture volatization losses which were observed to accompany sludge discharge, which will have increased the concentration of solids in these fractions. Total solids were observed to be concentrated in the retentate, relative to the permeate, which is consistent with the observations of Cheryan (1986) and Padilla and McLellan (1989). Ultrafiltration may remove colloidal macromolecules which would have been resistant to prior separation in the centifugation process on the basis of differences in density. This would be in agreement with Padilla and McLellan (1989) who expressed the opinion that the observed reduction in total solids by ultrafiltration is likely due to the removal of colloidal polysaccharides, proteins and large polyphenols. This opinion is supported by the work of Shieu et al. (1987), who found greater than 99% permeability of soluble solids in apple juice using a 50,000 MWCO membrane.

Similar to the results for total solids, a statistically significant difference was not observed between the centrifugal solids content (method described in section III-D) of the raspberry juice extract before and after centrifugation and ultrafiltration, although the mean values, 2.11 and 0.99 respectively, are suggestive of such a trend

TABLE 8. EFFECT OF CENTRIFUGATION AND ULTRAFILTRATION ON TOTAL SOLIDS AND CENTRIFUGAL SOLIDS IN RASPBERRY JUICE EXTRACT*.

Sample	Total Solids Content of Sample (%)	Centrifugal Solids Content of Sample (%)
Centrifuge feed	7.85 c*	2.11 c*
Centrifuge sludge	8.22 b	8.70 b
Secondary sludge (rerun of first sludge)	8.85 a	22.21 a
UF Retentate at VCR*** = 4	8.65 a	1.28 c
UF 50,000 MWCO permeate	7.77 c	0.99 c

Notes:

* Data from the single production run of July 12-14, 1988.

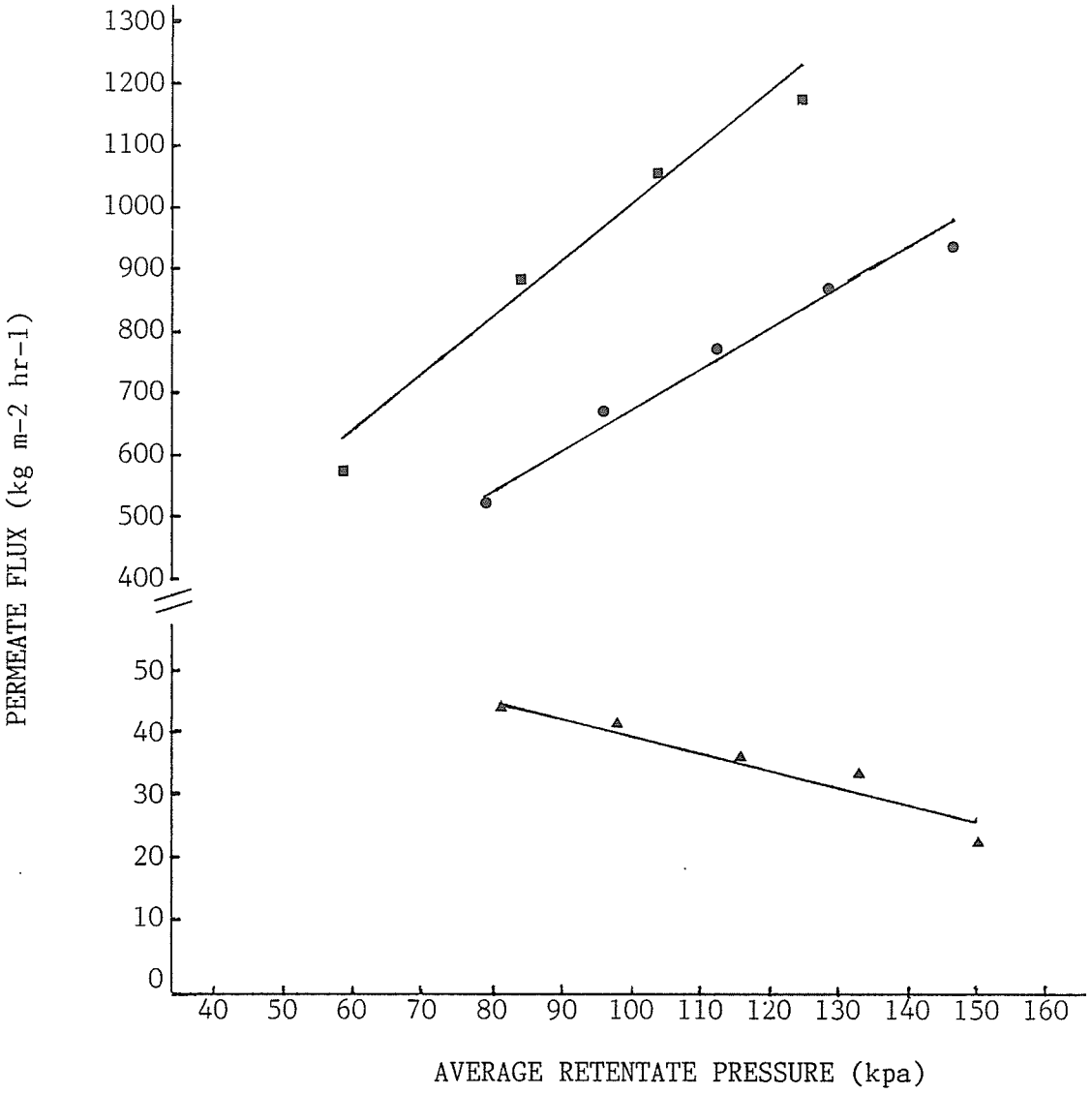
** Column values for means followed by the same letter are not significantly different ($P > 0.05$)($n = 4$).

*** Volume concentration ratio = (Initial feed volume)/(Remaining feed volume)

(Table 8). Similar to the effect on total solids, centrifugal solids were progressively concentrated in the centrifuge sludge fractions. Although the Westphalia continuous desludger centrifuge developed much higher relative centrifugal forces than did the laboratory centrifuge, it was unable to remove the residual 0.99% centrifugal solids found in the final ultrafilter permeate. The desludger centrifuge provided much less exposure time than did the laboratory centrifuge, and also had a continuous sweeping flow of the supernatant fraction through it, which may have carried with the supernatant some of the centrifugal solids later separated in the batch-type laboratory centrifuge.

3. ULTRAFILTRATION

Varying the retentate pressure in hollow fibre ultrafiltration alters the permeate flux. In the case of water (Figure 9), the effect of increasing the retentate backpressure by partially closing the retentate backpressure valve, such that the retentate pressure inside the hollow fibers at their outlet approaches that at their inlet, is to increase the flux through the membrane. This is to be expected as the total average pressure drop across the membrane would be increased, and is consistent with the observations of Finnigan and Lewis (1989) and Rao et al. (1987). Closing this valve also slows the water velocity within each hollow fibre, thereby reducing its momentum, allowing greater opportunity for the 90 degree change of direction required for crossing the membrane.



Notes:

Ave retentate pressure = (head pressure + back pressure)/2.

First water trial (●) was at 18.9°C, under same operating conditions as raspberry trial (▲) at 5.3°C. Later water trial (■) was at 3.9°C under changed operating conditions.

Plotted values represent only one replicate (n=1).

Regression and correlation:

■: Y = 77.4 + 8.97(X) ; r = 0.979

●: Y = 71.9 + 6.01(X) ; r = 0.993

▲: Y = 70.0 - 0.303(X) ; r = -0.951

FIGURE 9. RELATIONSHIP BETWEEN PERMEATE FLUX VS. AVERAGE RETENTATE PRESSURE FOR ULTRAFILTRATION OF WATER (■ , ●) AND RASPBERRY JUICE EXTRACT (▲).

For raspberry juice extract (Figure 9), however, the effect of increasing the retentate backpressure is opposite to the situation for water. Despite greater retentate pressure within the hollow fiber at the exit from the fiber, and correspondingly greater average pressure drop across the membrane, the permeate flux is reduced. This reduced permeate flux is attributed to the reduction in velocity of the retentate within the hollow fiber. Pepper (1987) stated the purpose of maintaining a high cross-flow velocity is to limit the build-up of material or fouling of the membrane surface. Because the membrane surface is continually being swept clean of colloidal substances, insoluble cellular debris and other particles, there is less tendency for fouling and greater opportunity for fresh retentate contact with the permeable membrane surface. These results for raspberry appear to differ slightly from the results obtained by Rao et al. (1987) for apple juice. Their work showed an increase in permeate flux when transmembrane pressures were increased from 105 to 140 kPa, above which permeate flux declined, which they attributed to possible collapsing of the fouling material which in turn closed membrane pores. The difference in the results might be attributable to probable differences in the nature of the suspended solids and colloidal macromolecules native to apple and raspberry juice extracts. It is possible, for example, that such compositional differences might result in compaction of these substances and resulting membrane surface fouling occuring more readily in the raspberry system. Rao et al. did not use centrifugation prior to ultrafiltration, but instead relied upon screening for particle removal on the basis of size, not density, which would also have

resulted in differences in the composition of the feed. Although the membrane used by Rao et al. was a Romicon model "PM50", nominally the same as used here, it appears to have been of a smaller configuration, having hollow fibres of a shorter length, which would have altered the relationship between average retentate pressure and retentate velocity, thereby altering the dynamics of membrane fouling.

4. TITRATABLE ACIDITY AND SOLUBLE SOLIDS IN CLARIFIED JUICE

Soluble solids in ultrafiltered raspberry juice extract processed from the 1986 and 1987 crops was within the range of 7.55% to 9.05% (Table 9). Titratable acidity ranged from 1.72% to 2.14%. There was variability between production runs, and soluble solids did not always vary in parallel with titratable acidity. The lots processed in July 1987 and February 1988 were entirely from raspberries which had been grown on a peat bog. Subsequent processing runs were done with mixtures of peat soil grown raspberries with mineral soil grown raspberries, and the January 1989 run only used raspberries which were grown on mineral soils. The typically greater availability of water to the raspberry plant under the peat bog production system may account for the lower concentrations of soluble solids and titratable acidity in the July 1987 and February 1988 production runs. The greater availability of water would have maintained greater turgor pressure in the peat grown berries, and thus greater dilution of the soluble solids and titratable acidity, especially in the sunlight hours during which plants typically experience a net loss of water to the atmosphere via evapotranspiration (Milthorpe and Moorby, 1974).

TABLE 9. TITRATABLE ACIDITY AND SOLUBLE SOLIDS IN ULTRAFILTERED RASPBERRY JUICE EXTRACT

Production Run	Crop Year	Titratable* Acidity (%)	Soluble** Solids (%)
29-July-87	1986	1.77 +/- 0.07	7.63 +/- 0.17
4-Feb-88	1987	1.72 +/- 0.04	8.18 +/- 0.05
16-June-88	1987	2.05 +/- 0.00	8.60 +/- 0.12
14-July-88	1987	2.00 +/- 0.04	7.55 +/- 0.13
29-Sept-88	1987	1.99 +/- 0.01	8.25 +/- 0.06
13-Jan-89	1987	2.14 +/- 0.05	9.05 +/- 0.06

Notes:
* Mean values +/- standard deviation (n=2)
** Mean values +/- standard deviation (n=4)

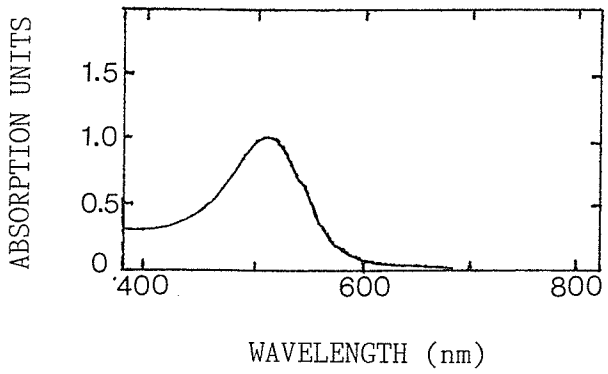
5. LIGHT ABSORPTION

Scanning spectrophotometer measurements of the light interaction properties of raspberry juice extract showed maximum absorptions at wavelengths of 514 nm, with complete transmission of the longer wavelengths associated with the human perception of red colouration. Only minor declines in maximum visible absorption at 514 nm occured as a result of ultrafiltration through the nominal 50,000 MWCO ultrafilter (Figure 10). At 1:20 dilution, absorption values at 514 nm for two samples of the feed were 0.971 and 1.007 units, compared to 0.868 to 0.937 units for two samples of the permeate, representing an average decline in absorption of 8.75%. The small molecular size of anthocyanins, between 440 and 770 molecular weight (Figures 2 and 3), relative to the nominal pore size of the ultrafiltration membrane suggests that a high degree of permeability of anthocyanins should be expected (Philip, 1984). The decline in absorption may be due to condensation of anthocyanins with tannins, macromolecules or cellular debris on the retentate side of the membrane (Markakis, 1982), either in the mobile recirculating retentate, or as part of the fouling of the membrane surface.

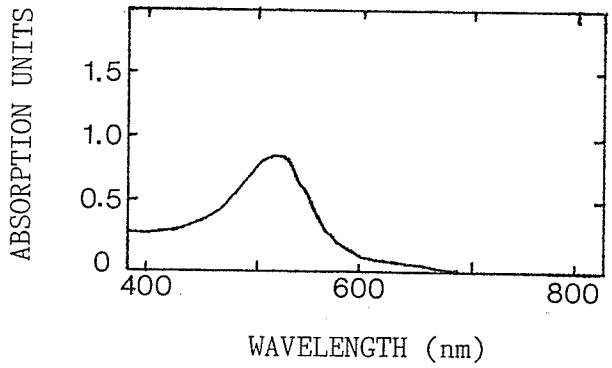
6. MICROBIOLOGICAL ANALYSES

The centrifugation process operation was effective in greatly reducing the level of viable yeast in raspberry juice (Table 10), but failed to remove other microorganisms. Yeast organisms may have been removed along with insoluble raspberry tissue debris and other high specific density substances, possibly ending up in the sludge fraction.

ULTRAFILTER FEED:



ULTRAFILTER PERMEATE:



Notes:
Samples dilution ratio: 1 volume in 20 volumes.
Wavelength of maximum visible light absorption: 514 nm.

FIGURE 10. RASPBERRY JUICE EXTRACT LIGHT ABSORPTION SPECTRUM FOR THE ULTRAFILTER FEED AND FOR THE ULTRAFILTER PERMEATE.

TABLE 10. EFFECT OF CENTRIFUGATION ON MICROBIAL CONTENT OF RASPBERRY JUICE.

Sample	SPC/ml*	Yeast/ml*	Mould/ml*
Centrifuge feed 1**	2400	380	100
Centrifuge feed 1	2600	560	50
Centrifuge feed 2	2300	20	10
Centrifuge feed 2	2600	10	20
Centrifuge product***	1900	<10	30
Centrifuge product	1900	10	10

Notes:

* Data accurate to two significant figures.

** Centrifuge feed was the crude press juice.

*** Feed samples 1 and 2 were combined as feed producing the centrifuge product.

The capability of ultrafiltration membrane processes to greatly reduce microbial contamination is expected. Having a nominal molecular weight cut-off of 50,000, which is equivalent to approximately 0.05 micron (Osmonics Inc, 1984), the ultrafiltration membrane used had a nominally smaller pore size than the 0.22 micron pore size generally considered adequate for bacterial, yeast and mould sterility. The presence of low levels of contamination in the collected permeate may be indicative of recontamination following ultrafiltration (Table 11).

The low pH level of the raspberry juice extract (Table 11) limits the potential for bacterial growth (Ayres, 1980).

7. COMPARISON WITH COMMERCIAL JUICES AND BEVERAGES

The raspberry juice extract, produced from Manitoba grown Boyne cultivar raspberry, can be regarded as comparable to the "100% pure raspberry juice" which has been available from Westvale Foods Ltd. (Chilliwack B.C.), with respect to analytical specifications (Table 12). The soluble solids and titratable acidity values are generally within the same ranges for the two pure juices, which predicts similar sweetness and sourness contributions for product formulation. Reported light absorptions at various wavelengths are also very similar, with the red value possibly being greater for the Boyne raspberry juice extract, which may indicate cultivar differences or possibly less pigment loss here due to the avoidance of heating during processing.

TABLE 11. EFFECT OF ULTRAFILTRATION ON MICROBIAL CONTENT AND pH OF RASPBERRY JUICE EXTRACT.

Sample	SPC/ml*	Yeast/ml*	Mould/ml*	pH
29-July-87				
UF Feed**	50	110	20	
UF Permeate	<10	<10	<10	3.0
6-Jan-88				
UF Feed	20	23	1	3.1
UF Permeate	<1	<1	<1	3.1
4-Feb-88				
UF Permeate	<10	<10	<10	3.1
17-Mar-88				
UF Feed	80	10	70	3.0
UF Feed	80	10	50	3.0
UF Permeate	<10	<10	<10	3.0
UF Permeate	<10	<10	<10	3.0
14-July-88				
UF Feed	280	130	20	3.3
UF Feed	320	90	10	3.3
UF Permeate	<10	<10	<10	3.3
UF Permeate	20	<10	<10	3.3

Notes:
* Data accurate to two significant figures.
** Ultrafilter feed was the centrifuge product.

TABLE 12. COMPARISON OF A COMMERCIALLY AVAILABLE PURE RASPBERRY JUICE VERSUS CLARIFIED BOYNE RASPBERRY JUICE EXTRACT.

Analytical Attribute	Westvale Raspberry Juice*	Clarified Boyne Raspberry Juice Extract
Brix**, Soluble solids	8.0° - 9.0° brix	7.5 - 9.1% sol. solids
pH	2.75 - 3.25	3.0 - 3.3
Tit. Acidity (citric)	1.7 - 2.3%	1.72 - 2.14%
Browning (abs at 430 nm)	< 0.4 (diluted 1:20)	approx 0.4 (1:20)
Red Value (abs at 520 nm)	0.6 - 1.0 (1:20)	1.0 at 514 nm (1:20)
Colour Ratio (520/430)	> 2.0	> 2.0
Turbidity (700 nm)	< 0.002 (1:20)	approx 0.0 (1:20)
Appearance	clear dark red	clear dark red
Yeasts / ml	< 100	< 10
Moulds / ml	< 100	< 10

Note:
* "Westvale 100% pure raspberry juice" as described in the Westvale Foods Ltd. specifications of June 15, 1984.
** Brix value was reported to have been determined by refractive index (H. Wiens, private communication, 1990).

A bright red raspberry juice beverage has been formulated by the author using the clarified raspberry juice extract processed here. Hedonic sensory evaluation of unidentified samples was requested of 93 shoppers in a major Winnipeg shopping mall. Their ratings indicated a significant preference for this product when compared against the two major similar products currently retailed in stores (Table 13). Verbal comments by the panelists typically indicated a preference for the flavor or aroma of the Boyne raspberry beverage. It is possible that avoidance of the use of elevated temperatures during processing was beneficial in this regard.

8. MARKET EXPERIENCE WITH CLARIFIED BOYNE RASPBERRY JUICE

The author has supplied raspberry juice extract, processed from raspberries grown on eleven Manitoba farms, to the University of Manitoba Food Science Department Dairy on a continuous commercial basis since October 1987, representing the first ever commercial processing of Prairie grown raspberry juice. The University Dairy then blends, pasteurizes and packages the product into cartons for refrigerated distribution to the cafeterias on campus. Priced the same as other juices, the product has captured sufficient market share to maintain its position on the shelf, generally paralleling apple juice sales, out-selling grapefruit juice and trailing orange juice. This is regarded as being successful for a non-traditional juice beverage.

TABLE 13. SENSORY ANALYSIS OF PREFERENCE FOR FORMULATED RASPBERRY JUICE BEVERAGES BY ADULTS OF CANADIAN PRAIRIES BACKGROUND.

Raspberry Beverage	Preference Rating*
Clarified Boyne raspberry beverage	2.86 a**
"TownHouse" brand raspberry beverage	1.87 b
"McCain" brand raspberry beverage	1.16 c

Notes:

* Ratings were on the hedonic scale:

+4 = like extremely
+3
+2
+1
0 = neither like nor dislike
-1
-2
-3
-4 = dislike extremely

** Mean values followed by different letters are significantly different ($P > 0.05$),(n = 93 panelists).

C. INVESTIGATION OF SUCROSE HYDROLYSIS IN RASPBERRY JUICE

1. PRELIMINARY OBSERVATIONS

Interest in the investigation of sucrose hydrolysis came about as a result of HPLC analyses for sugars in the clarified Boyne raspberry juice processed above. No sucrose was detected, and only fructose and glucose (together known as "invert sugar") were present in abundance in the clarified juice (treatment no. i, Tables 14-A and 14-B). This at first appeared to contradict the work of Lazzari (unpublished data, 1986), who had earlier analysed juice from fresh Boyne raspberries using the same HPLC equipment and proceedures, and found sucrose to comprise 38.7% to 43.2% of the sugars with fructose and glucose accounting for most of the remainder. Other researchers had also identified the presence of sucrose (Table 2). Sucrose inversion into fructose and glucose, at some time prior to the analyses conducted here, was a possible explanation for the different results.

To determine if sucrose inversion might have resulted from the processing of the clarified juice product using the above described operations (section III-D), which included the addition of a commercial pectinase mixed enzymes product and ultrafiltration, a nine kilogram sample of unadulterated raspberries from 1986 was allowed to partially thaw and the free-run juice from it directly analysed by HPLC. This non-processed juice, however, was also found to contain primarily fructose and glucose, with sucrose accounting for only 0.01% of the total major sugars (treatment no ii, Tables 14-A and 14-B). Work was therefore initiated to determine if sucose was indeed normally present

TABLE 14-A. OBSERVATIONS OF THE EFFECTS OF SEVERAL TREATMENTS ON THE INVERT SUGAR LEVEL AND pH OF RASPBERRY JUICE EXTRACTS.

No.	Treatment	Temp (°C)	Time (hours)	Invert Sugar (% of sugars)*	pH
i	87 Slow freeze, pectinase, UF	23	24.	100.	3.1
i	87 Slow freeze, pectinase, UF	23	24.	100.	3.1
i	87 Slow freeze, pectinase, UF	23	24.	100.	3.1
ii	86 Slow freeze	23	1.0	100.	
ii	86 Slow freeze	23	1.0	100.	
ii	86 Slow freeze	23	1.0	100.	
iii-a	88 Slow freeze	23	0.2	99.0	
iii-a	88 Slow freeze	23	0.2	99.3	
iii-b	88 Slow freeze, pectinase	23	0.2	97.9	
iii-b	88 Slow freeze, pectinase	23	0.2	99.9	
iv-a	Crush	23	0.2	70.9	3.1
iv-a	Crush	23	1.5	71.2	3.1
iv-a	Crush	23	4.5	80.5	3.1
iv-a	Crush	23	7.5	88.5	3.1
iv-a	Crush	23	10.5	94.7	3.1
iv-b	Pasteurize, crush	23	0.2	69.1	3.1
iv-b	Pasteurize, crush	23	1.0	69.1	3.1
iv-b	Pasteurize, crush	23	7.5	69.6	3.1
iv-c	Pasteurize, acidify, crush	23	0.2	70.0	2.6
iv-c	Pasteurize, acidify, crush	23	1.0	70.6	2.6
iv-c	Pasteurize, acidify, crush	23	7.5	69.9	2.6
v-a	Crush	23	0.2	69.7	3.1
v-b	Crush	23	1.0	72.3	
v-c	Crush	45	1.0	77.9	
v-d	Crush	55	1.0	78.0	
v-e	Crush	65	1.0	73.2	
v-f	Crush	75	1.0	74.7	
vi-a	Freeze, free-run	23	0.2	62.9	3.1
vi-a	Freeze, free-run	23	0.5	65.3	3.1
vi-a	Freeze, free-run	23	1.0	66.8	3.1
vi-b	Freeze, free-run, pectinase	23	0.5	81.7	3.1
vi-b	Freeze, free-run, pectinase	23	1.0	92.7	3.1
vi-b	Freeze, free-run, pectinase	23	1.6	94.9	3.1
vii	Immature, crush	23	0.2	80.4	

* % Invert = (glucose + fructose)/(sucrose + glucose + fructose) x 100

TABLE 14-B. OBSERVATIONS OF THE EFFECTS OF SEVERAL TREATMENTS ON THE RELATIVE LEVELS OF INDIVIDUAL SUGARS IN RASPBERRY JUICE EXTRACTS.

No.	Treatment	Temp (°C)	Time (hours)	Sucrose (% of total sugars)	Glucose	Fructose
i 87	Slow freeze, pectinase, UF	23	24.	0.0	45.3	54.7
i 87	Slow freeze, pectinase, UF	23	24.	0.0	45.2	54.8
i 87	Slow freeze, pectinase, UF	23	24.	0.0	44.5	55.5
ii 86	Slow freeze	23	1.0			
ii 86	Slow freeze	23	1.0			
ii 86	Slow freeze	23	1.0			
iii-a 88	Slow freeze	23	0.2	1.0	49.3	49.7
iii-a 88	Slow freeze	23	0.2	0.7	50.3	49.0
iii-b 88	Slow freeze, pectinase	23	0.2	2.1	48.8	49.1
iii-b 88	Slow freeze, pectinase	23	0.2	0.1	48.8	51.2
iv-a	Crush	23	0.2	29.1	35.7	35.2
iv-a	Crush	23	1.5	28.8	34.9	36.2
iv-a	Crush	23	4.5	19.5	39.9	40.6
iv-a	Crush	23	7.5	11.5	44.3	44.2
iv-a	Crush	23	10.5	5.3	48.5	46.2
iv-b	Pasteurize, crush	23	0.2	30.9	37.2	31.8
iv-b	Pasteurize, crush	23	1.0	30.9	38.3	30.8
iv-b	Pasteurize, crush	23	7.5	30.4	39.9	29.7
iv-c	Pasteurize, acidify, crush	23	0.2	30.0	36.5	33.5
iv-c	Pasteurize, acidify, crush	23	1.0	29.4	38.3	32.3
iv-c	Pasteurize, acidify, crush	23	7.5	30.1	35.7	34.2
v-a	Crush	23	0.2	30.3	31.3	38.4
v-b	Crush	23	1.0	27.7	31.7	40.6
v-c	Crush	45	1.0	22.1	36.1	41.8
v-d	Crush	55	1.0	22.0	34.6	43.4
v-e	Crush	65	1.0	26.8	33.0	40.2
v-f	Crush	75	1.0	25.3	33.1	41.7
vi-a	Freeze, free-run	23	0.2	37.1	31.0	31.8
vi-a	Freeze, free-run	23	0.5	34.7	31.8	33.5
vi-a	Freeze, free-run	23	1.0	33.2	33.1	33.7
vi-b	Freeze, free-run, pectinase	23	0.5	18.3	42.6	39.1
vi-b	Freeze, free-run, pectinase	23	1.0	7.3	54.1	38.6
vi-b	Freeze, free-run, pectinase	23	1.6	5.1	49.9	44.9
vii	Immature, crush	23	0.2	19.6	35.9	44.5

2. EFFECT OF TIME, PASTEURIZATION AND ACIDIFICATION

Treatments iv-a, iv-b and iv-c were replicated in triplicate and the samples held at 23°C with analyses conducted promptly within 0.2 hours of crushing, and again at 1.5, 3.0 and 4.5 hours. Although each replicate for the simple crushing treatment, iv-a., had a different level of invert sugar at 0.2 hour after crushing, the rate of increase in the level of invert sugar was similar for the three replicates. For example:

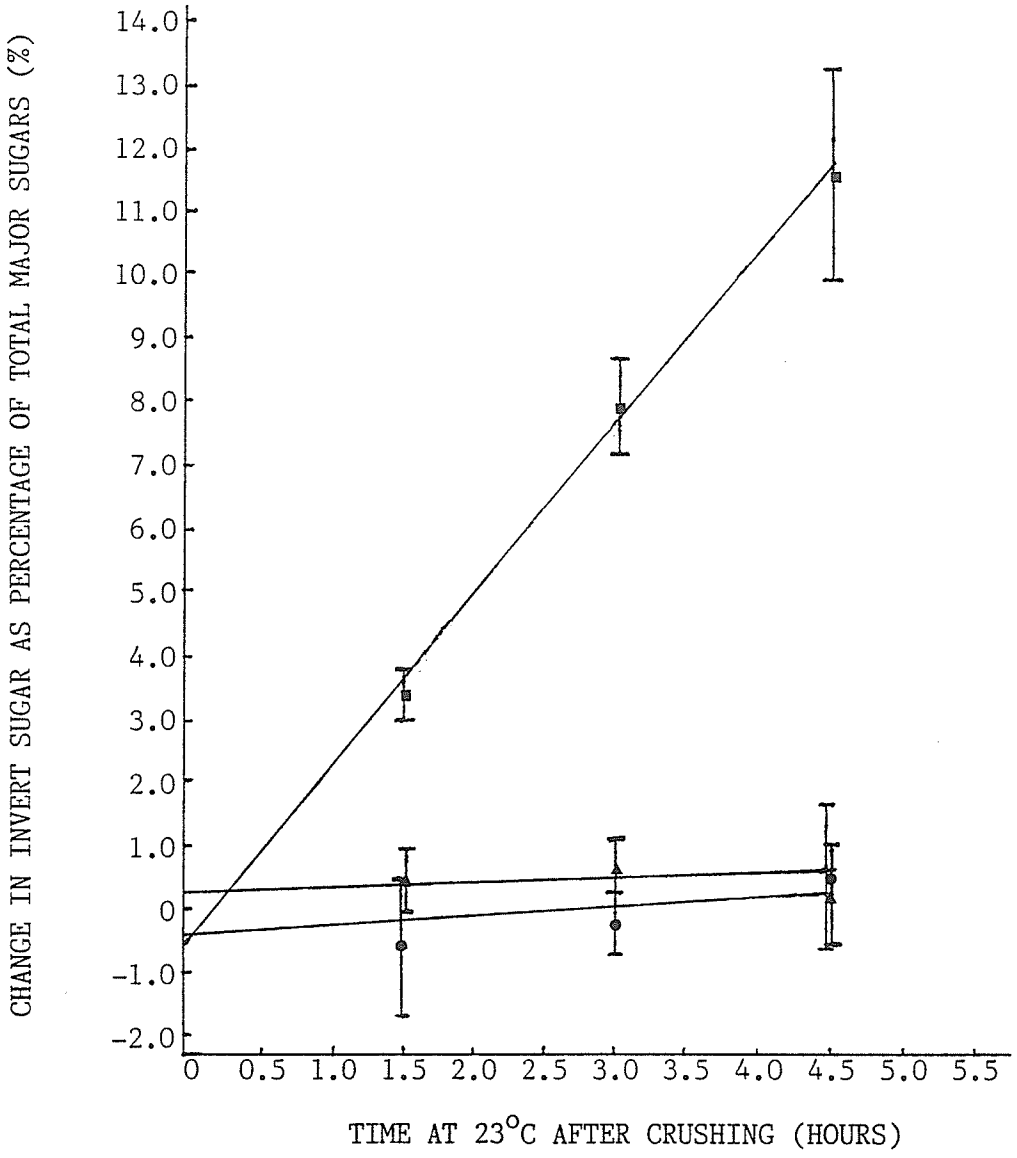
-the first replicate had 60.6% invert sugar at 0.2 hour, which increased by 8.6% to 69.2% at 3.0 hours of crushing;

-the second replicate had 67.5% invert sugar at 0.2 hour, which increased by 7.9% to 75.4% at 3.0 hours of crushing; and

-the third replicate had 84.2% invert sugar at 0.2 hour, which increased by 7.2% to 91.4% at 3.0 hours of crushing.

Change in percent invert sugar was therefore plotted as a function of time after crushing (Figure 11). This showed a relationship which can be described as linear within the range of observations made.

Replications of treatments iv-b. and iv-c., in which the fresh raspberries were subjected to a pasteurization treatment of 80°C for 30 seconds prior to being crushed, confirmed the preliminary observation that pasteurization prevents any further sucrose inversion. Pasteurization in effect stabilized the levels of sucrose, fructose and glucose (Figure 11). Lowering the pH from 3.1 to 2.6 by the addition of citric acid did not change the stabilizing effect of the pasteurization treatment. These results suggest that the sucrose inversion which has been observed to occur in crushed raspberry is not primarily due to the



Notes:

Mean values are plotted showing standard deviations (n = 3).

Mean values for crushing (■) are significantly different from each other, and also from all mean values for the other two treatments ($P > 0.05$). All mean values for treatments (▲) and (●) are not significantly different from each other ($P > 0.05$).

Regression and correlation:

■ : $Y = -0.52 + 2.70(X)$; $r = 0.988$

▲ : $Y = 0.24 + 0.0510(X)$; $r = 0.152$

● : $Y = -0.38 + 0.134(X)$; $r = 0.279$

FIGURE 11. EFFECT OF CRUSHING (■), CRUSHING AFTER PASTEURIZATION (▲), AND CRUSHING AFTER PASTEURIZATION AND ACIDIFICATION (●), ON THE HYDROLYSIS OF SUCROSE TO INVERT SUGAR IN RASPBERRY.

in Manitoba origin Boyne raspberries, and what conditions might be responsible for its inversion.

Treatment no. iii-a. similarly analysed Boyne raspberries which had been partially compressed into pails and frozen in bulk, with the differences being the year of harvest and the soil type. Sucrose was again found to be nearly absent, which eliminated both year and soil type as variables possibly responsible. The addition of the mixed pectinase enzymes product in iii-b., followed by prompt HPLC analysis, had no apparent impact on the already high level of invert sugar.

Treatment nos. iv., v., vi. and vii. used fresh 1990 Boyne raspberries for analyses. All twenty-four analyses confirmed the presence of substantial levels of sucrose in fresh Boyne raspberries, as had been established by Lazzari (1986). Simply crushing a sample of fresh raspberries, as in the no. iv-a. treatment, and then allowing the sample to stand at room temperature, suggested that inversion commenced with crushing and then progressed with time. After 10.5 hours most of the sucrose initially present had been hydrolysed into invert sugar. This appeared to explain the absence or very low levels of sucrose found in treatments i., ii. and iii., due to the raspberries analysed in those treatments having been effectively crushed when compressed into the pails and slowly frozen. The very low levels of sucrose in the pureed raspberry that Bettenfeld and Voilley (1983) studied supports the suggestion that disruption of cellular structure may be implicated in the absence of sucrose.

acidity of the system, but rather is due to the presence of catalysts which are heat labile. Such catalysts might be invertase enzymes of either plant, yeast or mould origin (Reed and Underkofler, 1966).

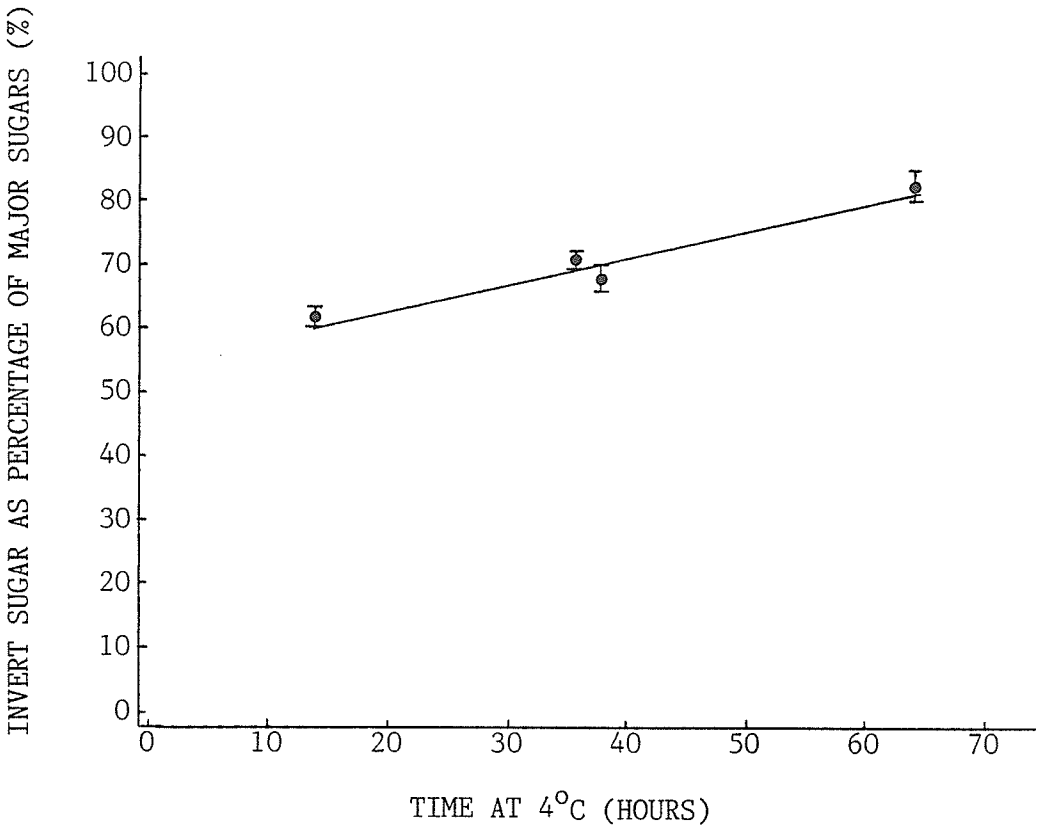
Further evidence that the catalysts might be enzymic in nature was provided by the observations made in treatments no. v-b. to v-f. (Tables 14-A and 14-B). The single replicate performed suggested the possibility of the rate of sucrose inversion being enhanced by increasing the temperature to 45°C and 55°C, but then declines at higher temperatures. If further replications had been possible, and they had confirmed this relationship, then it would indicate a temperature-activity curve which is typical of enzymes. Yeast invertase, which may be the enzyme involved here, has been reported to have an optimum temperature of 55°C, but begins to suffer denaturation at temperatures above 59°C (Reed and Underkofler, 1966).

If the primary catalyst is indeed an invertase enzyme, it is not clear whether or not it is of raspberry or microbial origin. Treatment vi-a. showed that the free-run juice resulting from freezing and thawing whole raspberries underwent inversion when placed at 23°C, similar to the effect seen for simply crushing fresh raspberries. A raspberry origin invertase might not leak readily from a thawing intact raspberry, and yeasts and moulds were determined to be present in the crude press juices previously analysed (Table 10 above).

The lower initial level of invert sugar at 0.2 hour for treatment vi-a when compared with treatments iv-a and v-a may be related to differences in time of storage of the whole fresh fruit at 4°C. Treatment vi-a had been placed at freezing temperature within seven

hours of harvest, whereas treatments iv-a and v-a were not initially analyzed until 36 and 21 hours respectively, after harvest. The data presented in Figure 12 shows that for fresh whole ripe raspberries, the proportion of the total major sugars present as invert sugar increased as a function of storage time at 4°C. This suggests that sucrose hydrolysis may be a metabolic activity in raspberry which is driven by native invertase enzymes. The data does not rule out, however, the possiblity of acid catalysed inversion occuring slowly within the vacuole of intact raspberry cells. As sucrose is the primary form in which energy is transported within plants (Raven et al., 1976), there is likely to be a mechanism within the raspberry fruit for the hydrolysis of the sucrose, which originated in photosynthetic organs, into the fructose and glucose which are present in the fruit. The analysis of one sample of immature raspberry in treatment vii. (Tables 14-A and 14-B) showing an invert sugar level of 80.4 %, suggests that such hydrolysis might also be occuring early in the development of the fruit.

Of particular interest with respect to clarified juice processing was the accelerated rate of inversion which occured when the commercial mixed pectinase enzymes product was added to the free-run juice in treatments vi-a. and vi-b. Whereas the invert sugar level had increased from the initial 62.9% by only 2.4% and 3.9% after 0.5 and 1.0 hours respectively for the two control treatments, the increase was 18.8% and 29.8% after 0.5 and 1.0 hours respectively for the pectinase-added treatments. An invertase activity thus appears to be present in the commercial pectinase product, which would have ensured the absence of



Notes:

Mean values are plotted showing standard deviations (n = 3).

Mean values at 36 and 38 hours are not significantly different from each other ($P > 0.05$). Mean values at 14 hours and 64 hours are significantly different from each other and from all other means ($P > 0.05$).

Regression and correlation:

$Y = 54.9 + 0.408(X)$; $r = 0.968$

FIGURE 12. EFFECT OF STORAGE AT 4°C ON THE HYDROLYSIS OF SUCROSE TO INVERT SUGAR IN WHOLE RASPBERRIES.

HPLC detectable sucrose in the clarified Boyne raspberry juice processed.

V. CONCLUSIONS

A. INVESTIGATION OF RASPBERRY JUICE EXTRACTION

The addition of a commercial pectinase-type mixed enzyme product prior to juice extraction resulted in increased crude juice extraction. A reaction time of 1.5 hours at 18°C was superior to 4.5 hours or 18.0 hours at 18°C with respect to total crude juice extraction, but resulted in a higher content of non-degraded macromolecules in the extracted juice than did the longer treatments. Using extreme physical maceration for the addition of the enzyme reduced crude juice extraction when compared with less aggressive manual stirring. Enzyme addition early during thawing resulted in potentially greater total clarified juice extraction than did allowing the raspberries to first thaw and warm to room temperature before enzyme addition.

B. CLARIFIED RASPBERRY JUICE PROCESSING TRIAL

The processing trial conducted, using the process operations of pectinase enzyme reaction, pressing, centrifugation and ultrafiltration, resulted in a 66.6 % recovery of the final clarified juice product, which was projected to be approximately 74.0 % for larger scale processing. Total solids were concentrated in the centrifuge sludge and also in the ultrafiltration retentate, relative to the ultrafiltration permeate. Increasing the average retentate pressure and

slowing its velocity within the hollow fibre, appeared to reduce permeate flux for raspberry juice extract, which supports the concept that a high cross-flow velocity minimizes fouling of the membrane surface. For six processing runs using 1986 and 1987 crops, titratable acidity values for the ultrafiltered raspberry juice extract ranged from 1.72 % to 2.14 % as citric acid, and soluble solids from 7.55 % to 9.05 % as invert sugar. Maximum visible light absorption occured at 514 nm with complete transmission of wavelengths greater than 700 nm. Ultrafiltration through the 50,000 MWCO membrane resulted in an average decline in maximum absorption of 8.75 %. The centrifugation step greatly reduced yeast contamination, and ultrafiltration removed nearly all microorganisms. The clarified Boyne raspberry juice extract processed was comparable to a commercially available pure raspberry juice with respect to its stated specifications, and a beverage formulated from it was found to be preferred by typical adult Winnipeg consumers when compared to the two major similar products currently retailed in stores. Boyne raspberry juice processed by this method has been supplied continuously for three years on a commercial basis to a dairy for usage in a juice beverage.

C. INVESTIGATION OF SUCROSE INVERSION IN RASPBERRY

Sucrose, fructose and glucose were found to be present in fresh Boyne raspberries, but sucrose was absent in the clarified juice extract processed. Sucrose was found to be slowly hydrolysed into invert sugar when fresh raspberries were stored at 4°C. Disrupting

tissue stucture, either by freezing and thawing or crushing, resulted in an increased rate of sucrose inversion at 23°C. Pasteurization at 80°C for 30 seconds stabilized the sugars at the initial levels, suggesting that the inversion otherwise observed might be due to heat labile enzymes of raspberry or microbial origin. The commercial pectinase-type mixed enzyme product was shown to also contain invertase activity.

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APPENDIX A

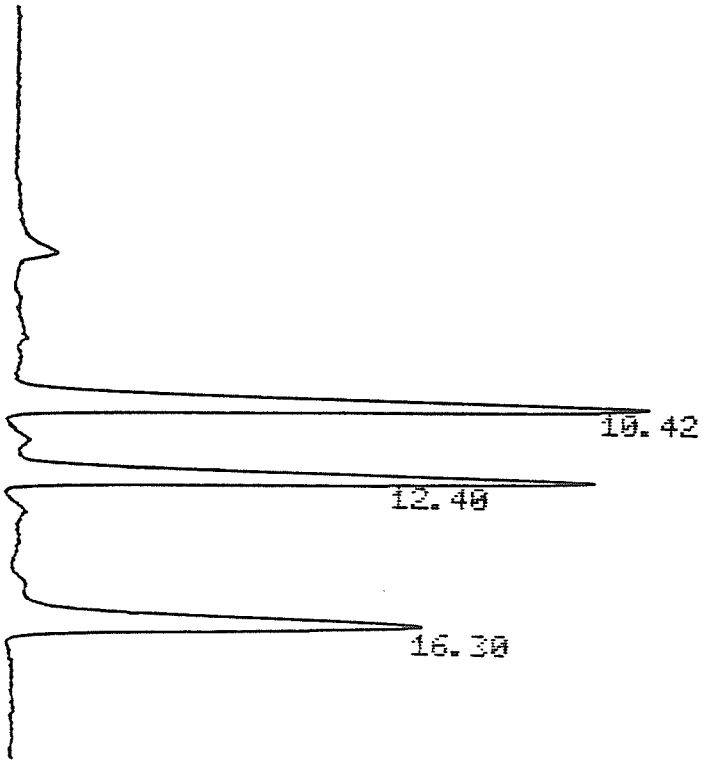

FIGURE 13. HPLC CHROMATOGRAM OF MAJOR SUGARS IN FRESH RASPBERRY IMMEDIATELY AFTER CRUSHING. SUGAR CONCENTRATIONS ARE RELATED TO THE PEAK AREAS: SUCROSE AT 10.42 MINUTES, GLUCOSE AT 12.40 MINUTES AND FRUCTOSE AT 16.30 MINUTES.

APPENDIX A (cont'd)

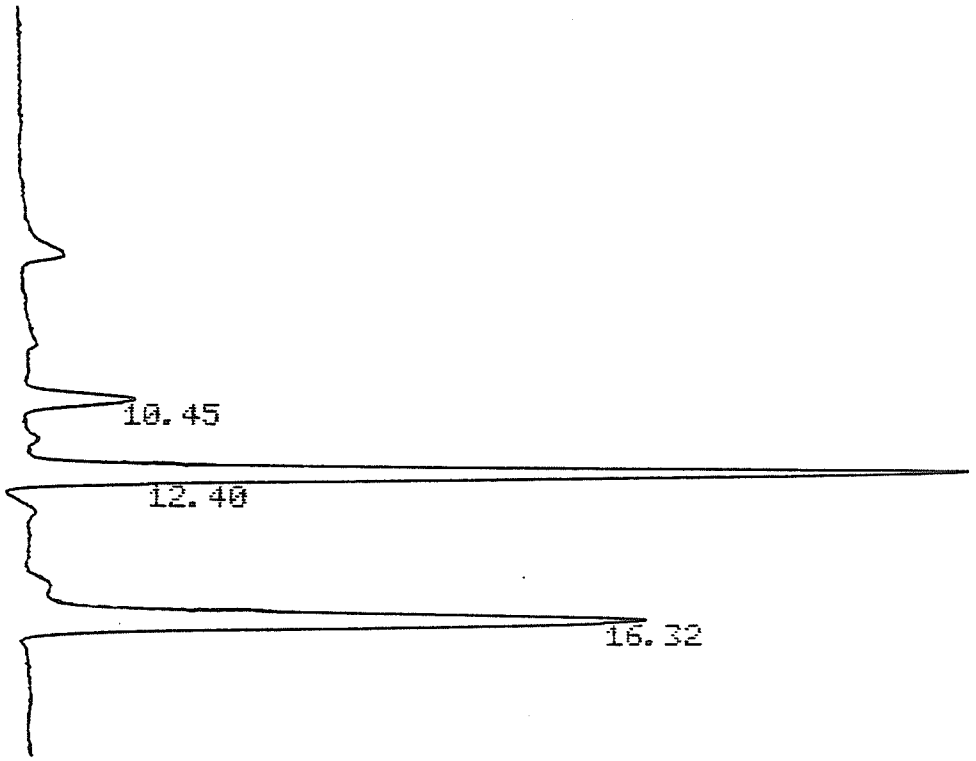

FIGURE 14. HPLC CHROMATOGRAM OF MAJOR SUGARS IN FRESH RASPBERRY AFTER CRUSHING AND STORAGE AT 23°C FOR 10.5 HOURS. SUGAR CONCENTRATIONS ARE RELATED TO THE PEAK AREAS: SUCROSE AT 10.45 MINUTES, GLUCOSE AT 12.40 MINUTES AND FRUCTOSE AT 16.32 MINUTES.

APPENDIX B-Sensory Ballet

CONSUMER EVALUATION OF RASPBERRY BEVERAGES
October 30, 1987. Polo Park Shopping Centre.

INSTRUCTIONS:

Please sip a small portion of your first sample, and then indicate how much you like or dislike it.

Likewise for the second and third samples.

If your are then uncertain of any evaluation, please repeat in the order of your choice.

Sampling order: First " ", then " ", and then " ".

EVALUATIONS:

Please circle the number indicating your opinion.
"-4" corresponds to "dislike extremely"
"0" corresponds to "neither like nor dislike"
"+4" corresponds to "like extremely"

Sample :

-4 -3 -2 -1 0 +1 +2 +3 +4
(dislike) (like)

Why did you assign this rating?

Sample :

-4 -3 -2 -1 0 +1 +2 +3 +4
(dislike) (like)

Why did you assign this rating?

Sample :

-4 -3 -2 -1 0 +1 +2 +3 +4
(dislike) (like)

Why did you assign this rating?

APPENDIX B-Sensory Ballet (continued)

May we ask you for some personal data?

Frequency of fruit beverage consumption: (please check category)

___ seven or more times per week.
___ one to seven times per week.
___ less than one time per week.

Mainly which juices?

Age category:

___ under 18.
___ 18 to 29.
___ 30 to 49.
___ 50 and over.

Sex:

___ female
___ male

Background:

How would you describe the cultural background in which you were raised?

(For example: "Winnipeg", "Rural Manitoba, Ukranian", "Quebec", "Mexico", etc.)