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High pressure processing of Australian navel orange juices: Sensory analysis and volatile flavor profiling

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Abstract

Navel orange juices subjected to high pressure processing (HPP) and temperature treatment (TT) were stored at 4 and 10 °C for up to 12 weeks to establish the shelf-life of such products. The processed juices and a control juice, stored at −20 °C, were assessed by a trained sensory panel and a consumer acceptance panel at 0, 1, 2, 4, 8 and 12 weeks or until such time that the juices were considered unfit for consumption. Untreated juice stored at 4 °C was similarly assessed for up to 2 weeks and untreated juice stored at 10 °C was assessed for up to 1 week. The volatile components of corresponding juices were isolated by SPME and the extracts were analyzed by GC–MS. Twenty key aroma compounds were selected for quantification and these data were used to monitor the change in volatile content of the juices during storage. The study showed that the odor and flavor of the HPP juice was acceptable to consumers after storage for 12 weeks at temperatures up to 10 °C. However, only the TT juice stored at 4 °C was acceptable after the same length of storage.

Keywords: Orange juice; Sensory analysis; Consumer acceptance; Shelf-life; Odor; Flavor; GC–MS; SPME

Industrial relevance: Orange juice is a sensitive product subject to a high microbial load that can tolerate only moderate heat treatment without the destruction of the product's delicate aroma and flavor characteristics. High pressure processing at moderate pressures and storage at refrigeration temperatures have been evaluated as means of maximizing microbial inactivation while maintaining consumer acceptability of the product. The sensory and analytical data presented demonstrate that high pressure processing with refrigeration can extend the shelf-life of orange juice while maintaining consumer acceptability.

1. Introduction

Previous studies have identified many volatile compounds that are important for good orange juice aroma and flavor (Bettini, Shaw & Lancas, 1998; Petersen, Tønder & Poll, 1998; Shaw, 1991; Dürr & Schobinger, 1981) and odors and off-flavors associated with ageing of the juice. Typically the results obtained by gas chromatography (GC) were correlated to those from trained sensory panels for the purposes of evaluating changes due to storage and/or temperature conditions (Bettini et al., 1998; Petersen et al., 1998; Tønder, Poll & Petersen, 1996; Vélez et al., 1993; Naim, Striem, Kanner & Peleg, 1988; Guadagni, Bomben & Mannheim, 1970). None of the aforementioned studies have included consumer panels, so often trained or semi-trained sensory panelists have made judgments on the 'freshness' and acceptability of the samples. However, by merit of their training, sensory panelists are not representative of the average consumer and, as panelists are not necessarily regular consumers of the product, they may not be part of the target population to which the hedonic results would be generalized. A search of the literature did not yield any studies combining the results of GC–MS analysis of orange juice volatiles with both sensory and true consumer acceptance panels. Thus the study reported here provides a unique understanding of the sensory and chemical factors influencing the shelf-life of orange juice processed and stored under different conditions.

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High pressure processing (HPP) has been reported to reduce bitterness in orange juice, while having no adverse effect on color or texture, with HPP orange juice reportedly preferred over a commercial brand of freshly squeezed orange juice and demonstrating a 16-week shelf-life at 4–8 °C (Anonymous, 1996). The potential de-bittering effect of HPP is of interest as Navel orange juice is known to develop bitter notes (Chandler, 1971). Another study found no difference between HPP orange juice and a reference sample in terms of sensory quality when kept chilled for up to 20 weeks (Takahashi, Pehrsson, Rovere, & Squarcina, 1998).

The aim of this study was to determine the shelf-life of Australian Navel orange juice processed by HPP and by conventional thermal treatment (TT) when stored under refrigerated (4 °C) and temperature-abuse (10 °C) conditions. Additionally, the authors wanted to understand the effects of processing and storage on the volatile flavor/aroma composition and sensory character of the juices.

2. Materials and methods

2.1. Chemicals and solvents

All reagents used in the preparation of the simulated orange juice solution were purchased from Sigma Chemical Co., (St. Louis, MO). Reference samples of orange juice odor-active compounds were purchased from three suppliers, Fluke Chemie GmbH (Buch, Switzerland), Sigma Chemical Co. (St. Louis, MO) and Aldrich Chemical Co., Inc. (Milwaukee, WI). All compounds had a purity >98%. Distilled water was further purified by filtration through a Milli-Q filtration system (Millipore Corp., Bedford, MA).

2.2. Product

The Navel oranges were purchased from a local fruit market in Sydney, New South Wales. The oranges were harvested in Mildura, 1027 km south–west of Sydney and were of a "good"-grade quality. The Navel trial oranges were stored without refrigeration for 2 days prior to processing: the ambient temperature at that time of the year was approximately 15 °C.

2.3. Preparation and processing of oranges

All equipment used in the project was sanitized prior to use by washing with a 100 mg/l sodium hypochlorite/water solution followed by a water rinse. Any damaged fruit was removed. The oranges were soaked in a 100 mg/l sodium hypochlorite/water solution for 2 min and rinsed in water. Oranges were hydraulically pressed with an industrial juice extractor (FMC-Corporation, USA) and the peel, rind and excess pulp were automatically discarded. The juice was strained through a 1 mm mesh and collected into large stainless steel containers. The untreated juice and juice to be pressure-treated were immediately distributed into 200 ml polyethylene terephthalate (PET) bottles with screw cap closures (ACI Petalite, Australia) using an automated foot-controlled bottler (Micro-Packaging Engineers Ltd, Australia).

A 2 l processing unit (Flow International Corporation, USA) was used to pressurize the orange juice samples at 600 MPa for 60 s. The pressure fluid was water, come up time to the designated pressure was less than 10 s, and depressurization was less than 5 s. Pressurization was carried out at ambient temperature (18–20 °C), however, chilling the samples to 4 °C before pressure treatment minimized the effects of adiabatic heating during pressurization.

An in-house, custom-built 2 l thermal pasteurizer with a holding tube and cooler was used to indirectly steam heat the samples. The Navel juice was pasteurized at 85 °C for 25 s and distributed into bottles directly from the pasteurizer outlet.

2.4. Storage and sampling of orange juice

After processing, the juice was stored in two separate controlled-temperature rooms at approximately 4 and 10 °C, with temperature measurements taken at 30-min intervals using calibrated Tinytag™ temperature data loggers (Gemini Dataloggers, UK).

The samples evaluated by sensory analysis and flavor profiling were: an untreated orange juice stored at −20 °C (U −20 °C; the control), untreated samples stored at 4 and 10 °C (U 4 and U 10 °C, respectively), thermally treated samples stored at 4 and 10 °C (TT 4 and TT 10 °C, respectively), and high pressure processed samples stored at 4 and 10 °C (HPP 4 and HPP 10 °C, respectively). Due to increased microbial activity or unpalatability (as judged by the sensory scientist) not all samples were evaluated throughout the 12-week trial. Table 1 shows the Navel juice samples that were tested and found to be acceptable for inclusion in the sensory evaluation at each time point.

Table 1
Australian Navel orange juice samples included in sensory testing during weeks 0–12

Sample	Week 0	Week 1	Week 2	Week 4	Week 8	Week 12
U −20 °C[a]	✓	✓	✓	✓	✓	✓
U 4 °C	✓	✓	✓			
U 10 °C	✓	✓				
TT 4 °C[b]	✓	✓	✓	✓	✓	✓
TT 10 °C	✓	✓	✓	✓		
HPP 4 °C[c]	✓	✓	✓	✓	✓	✓
HPP 10 °C	✓	✓	✓	✓	✓	✓

[a]U=untreated juice; [b]TT=temperature treated juice; and [c]HPP=high pressure processed juice.

Samples were sensory tested (trained panel and consumer evaluation) only after microbiological safety clearance (Bull et al., 2004). Sensory evaluations were conducted at baseline (2 days after processing) and then at weeks 1, 2, 4, 8 and 12 of shelf-life. Isolation and analysis of volatile compounds using GC–MS commenced at the same time as the sensory analysis.

The total volume of sample required for each day of sensory testing was removed from the appropriate storage rooms 2 h prior to the first session. The frozen control samples were defrosted under running cold water. The total volume of each sample was decanted into 2 l lidded plastic jugs labeled with blind codes and stored in the refrigerator until required. Prior to commencing sensory sessions, the samples were removed from the refrigerator and stored on ice to keep them cool. As soon as the session had finished, the samples were placed back in the refrigerator.

2.5. Panel training

The sensory panel, comprising 10 sensory panelists experienced in the technique of Quantitative Descriptive Analysis® (QDA; Stone, Sidel, Oliver, Woolsey & Singelton, 1974; Stone & Sidel, 1992), completed 18 h of training as per the QDA technique. Eight hours were spent developing the orange juice descriptors (product vocabulary) to be used for evaluation of the Navel orange juice and agreeing upon a standard sample assessment procedure. Further 6 h were then spent scoring the orange juices on 100 mm line scales, presented using Compusense® (sensory software; Compusense®, Guelph, Canada), and redefining the vocabulary as required. Finally, two 2-h sessions were held to evaluate the panel's performance prior to the Navel shelf-life study beginning. Table 2 shows the descriptors used by the trained panel for the Navel orange juice study.

The panelists were trained using four orange juice samples: U −20 °C (defrosted 3 h prior to the session), a HPP 4 °C sample held at this temperature for between 4 and 10 days, a TT 4 °C sample held at this temperature for 4–10 days, and an untreated juice sample stored at 30 °C (U 30 °C) for 2–5 days. All juices, except the U 30 °C sample, were microbiologically cleared prior to assessment. The U 30 °C sample was assessed for appearance and aroma only (i.e. panelists were instructed not to drink this sample) to familiarize the panel with sensory characteristics that might be expected as the juices aged in the shelf-life trial.

The training samples were evaluated in duplicate over two consecutive days in the sensory laboratory to assess panel performance. The evaluation was conducted using a balanced sample presentation order to minimize bias due to first-order and carry-over effects (MacFie, Bratchell, Greenhoff & Vallis, 1989). Repeated measures analysis of variance (ANOVA) was used to analyze the data to show any panelists/sample/session effects and also to ensure that the product descriptors to be used discriminated between the samples.

Table 2
Vocabulary developed to describe Australian Navel orange juice

Attribute	Definition
Appearance	
Color	Depth of color. Judged by looking from the top of the cup.
Aroma	
Sweetness	The typical odor of sugary drinks.
Aged	The odor associated with ageing oranges (bruising).
Artificial	Odor of orange essence, as associated with orange cake.
Fermented	Yeasty, fruity, fermented odor; wine-like.
Overall strength of orange aroma	Overall intensity of the orange aroma.
Flavor	
Sourness	Typical 'tangy' sourness, perceived at the side of the tongue.
Sweetness	Immediate perception of sweetness.
Processed	Artificial taste associated with heat treatment ('cooked' taste).
Bitterness	A bitter sensation associated with the oils extracted from the peel. Perceived towards the back of the throat.
Overall strength of orange flavor	Typical flavor associated with fresh orange juice.
Aftertaste	
Acidity	A tangy acidity at the back of the mouth. Gives the sample 'bite'.
Sourness	A lingering sensation of sourness, perceived at the side of the tongue. Has a mouth-puckering effect.
Bitterness	Bitter sensation perceived at the back of the throat after swallowing.
Duration	The length of time that the overall aftertaste remains up to 1 min after swallowing.

2.6. Descriptive sensory analysis of the samples

The trained panelists evaluated the test samples in duplicate in the morning over two consecutive days. All evaluations took place under controlled sensory conditions (e.g. individual booths, 3-digit sample blinding codes) using Compusense® for sample presentation and data capture. A complete block design was used and samples were presented in a balanced order.

Samples were evaluated for the 15 product descriptors (Table 2) using 100 mm line scales anchored at 0 mm with "weak" and at 100 mm with "strong". Palate cleansing breaks between every sample (using plain white bread and water over a 60 s interval) were enforced. Panelists were given a 10-min break after every fourth sample to minimize sensory fatigue.

2.7. Consumer acceptability

Between 30 and 40 regular consumers of freshly squeezed orange juice participated in each consumer evaluation session, held in the sensory laboratory immediately after the trained panel evaluations. All consumers were

aged between 18 and 65 years of age and an approximately equal split of males to females was recruited for each session. Samples were labeled with 3-digit blinding codes and presented in a balanced order, with all data collected using Compusense®. All samples were tasted in one session.

The consumers scored the acceptability of the samples using a 9-point hedonic category scale (where 1=dislike extremely and 9=like extremely), rating appearance, aroma, flavor, texture, aftertaste and overall acceptability (Meilgaard, Civille, & Carr, 1991). Consumers were also asked to provide comments on each sample to explain why they liked or disliked the sample.

2.8. Isolation of volatile compounds for GC–MS analysis

Samples of orange juice for instrumental analysis were removed from the constant temperature rooms at the same time as those were taken for sensory evaluation. These analytical samples were immediately stored at −20 °C. Analysis of the juice was conducted either on the same day or the following morning.

Samples of frozen juice were thawed and gently shaken to disperse the solids. The juice (950 ml) was placed in a centrifuge tube and the solid material was separated from the liquid by centrifugation at 6000 rpm for 10 min, according to the method of Steffen and Pawliszyn (1996). A sample (20 g) of the resulting supernatant liquid was placed in a 40 ml headspace vial (Alltech Associates Inc, Deerfield IL) containing a stirrer bar and sodium chloride (6 g).

For quantitative analysis of selected compounds 50 μl or 25 μg of an internal standard, 4-methylpentanol, was added to this solution (Tønder, Petersen, Poll & Olsen, 1998). The vial was capped with a Teflon-lined septum and the contents were vortexed for 2–3 min to dissolve the sodium chloride. The resulting liquid was equilibrated in a water bath (40 °C) for 10 min and the headspace above the liquid was sampled using a solid phase microextraction (SPME) fiber (carboxen/polydimethylsiloxane, 75 μm, Supelco, Bellefonte, PA) according to the method of Steffen and Pawliszyn (1996). After sampling the headspace volatiles for 1 h, the fiber was retracted into its sheath and then immediately transferred to the injector port of a gas chromatograph–mass spectrometer (GC–MS).

2.9. Analysis of volatile compounds by GC–MS

Gas chromatography was performed on an Alltech AT-5 fused-silica glass capillary column (60 m × 0.25 mm; 1 μm film thickness: Alltech Associated Inc., Deerfield, IL) housed in a HP5890 series II GC (Hewlett Packard, Palo Alto, CA) fitted with an electronic pressure control unit. Helium was used as the carrier gas at a constant flow of 1 ml/min after an initial pressure of 190 psi for 9 min. The oven temperature was initially set at 30 °C and, after 1 min, was heated to 195 °C at 3 °C/min and then ramped at 30 °C/min to 285 °C. The final oven temperature of 285°C was maintained for 1 min. The volatile compounds were desorbed by exposing the fiber to the environment of the injector port, which was set at 230 °C and operated in the splitless mode, whilst the first centimeter of the GC capillary column was cooled to −60 °C using liquid carbon dioxide. After 1 min, the purge valve was activated, cooling was stopped and the cooled section was ballistically heated to 250 °C. The capillary column was directly interfaced to an HP 5972 mass selective detector (MSD) that was operated in the electron impact and scan acquisition (30–400 amu) modes. The energy of the electron beam was 70 eV. The ion source temperature was 180 °C and the transfer line was set at 285 °C. The GC and MSD were controlled by a Hewlett Packard MS-ChemStation running on a Hewlett Packard Vectra VL2 computer. The data were acquired, stored and processed by the same station. The mass spectra of the individual components were matched with reference spectra in the NIST/EPA/NIH and Wiley mass spectral databases. Confirmation of identification of selected compounds was achieved by comparing linear retention indexes (LRI) with those of authentic compounds.

2.10. Determination of relative concentrations of selected compounds

The concentrations of selected compounds present in the juice samples were determined using the method of Jia, Zhang and Min (1998). The method was modified by the use of a sugar, acid and pectin solution in place of deodorized juice as solvent for the calibrant solutions. This solution was prepared from glucose (20.3 g), fructose (24.8 g), sucrose (48.1 g), malic acid (2.0 g), citric acid (9.3 g) and pectin (1.3 g) dissolved in purified water (1 l). The range of concentrations for the calibrant solutions of each compound was estimated from concentrations reported by Shaw (1991) and Chen, Shaw and Parish (1993) and from our own preliminary work. The range of concentrations varied greatly, for ethyl propanoate from 0.1 to 7 μg/ml and for limonene from 10 to 110 μg/ml. Five concentrations were prepared to cover the appropriate range for each compound. The concentration of the internal standard was constant and the same as that used in the analysis of the juice samples. Quantitation was performed by data system integration of the total ion chromatograms by recording the area counts under each peak. The calibration lines were obtained by plotting the ratio of compound area and internal standard area against compound concentration. Where necessary these lines were forced through zero. Analysis of duplicate samples of fresh and processed juices showed that the reproducibility of the method varied between 1% and 10% for the 20 selected compounds. The detection limit of the method was 0.001 μg/ml based on a factor of 3× background noise.

2.11. Analysis of volatile compounds absorbed by PET bottles

Three PET bottles used for the storage of control juice samples for 12 weeks were emptied of their contents, washed with purified water and allowed to drain for 2 h. The dry bottles were cut into strips (2 × 1 cm) and the strips (2 g) were placed in 40 ml headspace vials. The vials were capped and the vials and contents were heated at 50 °C in a water bath. The headspace volatiles were sampled by SPME for 1 h and the absorbed compounds were transferred to the GC–MS. These compounds were then analyzed using the same conditions as previously described for the analysis of the orange juice extracts (Section 2.9).

2.12. Data analysis

Analysis of variance (ANOVA; Minitab v13.31) was conducted on the data from the trained panel evaluations to determine samples that differed significantly ($p < 0.05$) at each time point for particular sensory attributes. The data from the consumer acceptability evaluations were also analyzed using ANOVA. Tukey's test (at the 5% significance level) was used once ANOVA showed significant differences to determine how the samples differed.

To illustrate the relationship between the sensory characteristics of the juice samples and consumers' overall acceptability, an external preference map of the samples at weeks 1 and 12 of the storage trial was constructed. This correlation between the consumers' mean overall liking score and the panel's sensory profiling data was determined by partial least squares type 1 regression (PLSR1; Unscrambler v 8.0, Camo A/S, Oslo, Norway). All data were standardized (1/standard deviation) prior to the PLSR1 analysis to prevent differences in scale usage affecting the loading given to each attribute.

PLSR1 was also used to model the relationship between the individual sensory attributes and GC–MS volatile compounds. The chemical data set were the *X*-variables (independent variables) and the individual sensory attributes formed the *Y*-variables (dependent compounds). Full cross validation was selected as the validation model, using the optimal number of principal components. The Martens uncertainty test (http://www.camo.com/pdf/Martens.pdf) was used to identify significant *X*-variables.

3. Results and discussion

3.1. Trained panel

Fig. 1 and Tables 3 and 4 show the mean trained panel scores by attribute for each of the samples examined at each time point. An overall trend was observed in the samples whereby, at week 4, the odor, flavor and aftertaste attributes significantly increased for most samples then decreased

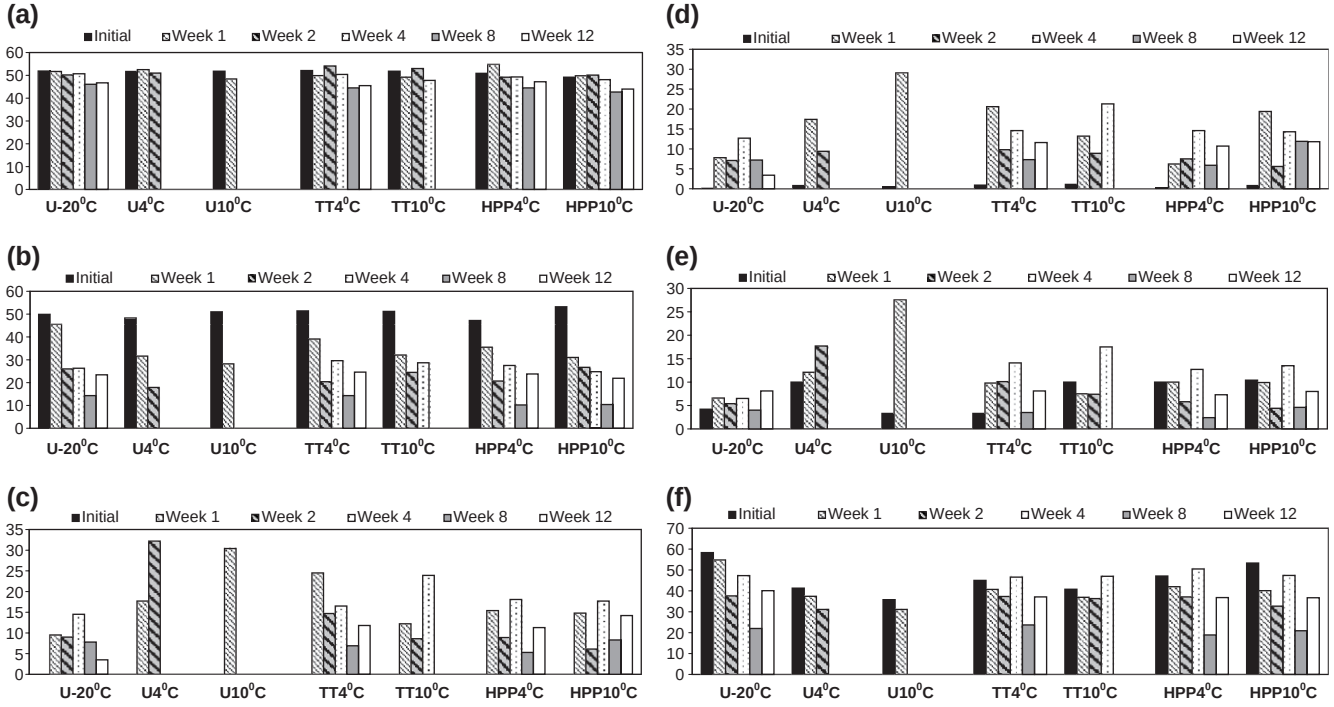

Fig. 1. Sensory panel scores for Navel orange juice appearance (color) and odor attributes over 12 weeks storage: (a) color, (b) sweet odor, (c) aged odor, (d) artificial odor, (e) fermented odor, and (f) strength of orange odor.

Table 3
Sensory panel scores for Navel orange juice flavor attributes over 12 weeks storage

Attribute	Time	Juice samples						
	(Week)	U −20 °C[a]	U 4 °C	U 10 °C	TT 4 °C[b]	TT 10 °C	HPP 4 °C[c]	HPP 10 °C
Sour taste	0	39.5	40.1	50.4	37.0	38.9	31.9	31.3
	1	33.5	27.5	40.3	39.6	34.9	41.3	42.2
	2	23.8	23.2		24.6	25.4	28.3	27.8
	4	45.9			28.6	33.5	30.2	39.9
	8	23.2			10.8		16.3	23.0
	12	19.2			15.9		15.7	17.2
Sweet flavor	0	53.6	47.3	41.8	52.4	56.2	47.2	48.8
	1	42.6	33.7	27.6	37.7	34.5	34.8	37.6
	2	28.1	30.9		33.8	33.4	29.4	28.4
	4	28.0			45.6	46.4	35.5	27.9
	8	30.6			36.2		42.1	30.0
	12	34.9			38.4		38.3	38.1
Processed flavor	0	8.8	20.4	2.1	7.5	29.2	10.8	7.1
	1	11.4	29.4	51.6	24.1	26.9	24.9	25.8
	2	12.7	21.9		13.8	15.7	11.4	13.8
	4	29.5			30.7	40.4	26.0	29.1
	8	22.9			15.3		24.7	21.1
	12	23.1			17.6		16.6	18.3
Bitterness	0	15.3	17.3	13.8	19.1	19.2	16.9	19.6
	1	15.1	28.3	51.7	29.6	29.3	23.9	24.4
	2	19.9	24.1		19.2	22.9	25.9	23.0
	4	48.8			38.9	31.1	35.5	46.1
	8	26.5			12.6		16.5	22.5
	12	23.2			18.2		16.8	18.1
Strength of flavor	0	66.3	41.3	45.4	60.8	59.6	49.9	53.8
	1	56.4	39.6	28.8	48.3	41.3	47.5	42.8
	2	46.4	38.2		42.5	41.8	45.8	40.6
	4	48.1			54.6	48.1	53.5	49.9
	8	31.8			32.1		34.6	32.9
	12	41.6			38.9		38.2	38.7

[a]U=untreated juice; [b]TT=temperature treated juice; and [c]HPP=high pressure processed juice.

significantly at week 8. This trend was also observed in the data on volatile composition (Section 3.4). The *TT 10 °C* sample was found to be unacceptable for consumption by the trained panel at week 8 so there are no further sensory data available on this sample.

3.1.1. Appearance and odor attributes

Mean panel scores for appearance and aroma are shown in Fig. 1. There were no significant differences between samples with regard to the juice *color* at any given time point (i.e. comparing mean scores for all samples at one time point using ANOVA). There were no significant changes in the color of any sample over the course of the 12-week shelf-life.

By week 1, the U 10 °C sample had a less intense *sweet odor* and weaker overall *strength of orange odor* than the U −20 °C (control) and processed samples. The U 10 °C juice was however the sample with the strongest *aged odor*, *artificial odor* and *fermented odor*. The TT and HPP samples were overall similar to each other with regard to odor characteristics.

Generally, increased storage time lead to a decrease in *sweet odor* and *strength of the orange odor* and an increase in intensity of *aged odor*, *artificial odor* and *fermented odor* across all the test samples. However, because the presence of a strong fermented odor would indicate microbial spoilage, samples displaying this characteristic were not tasted by either the panel or consumers.

3.1.2. Flavor attributes

Mean panel scores for Navel orange juice flavor attributes are shown in Table 3. By week 1, the U 10 °C sample had a stronger *sour*, *bitter* and *processed flavor* as compared to the other samples and was the least *sweet* sample and lowest in *overall strength of orange flavor*. The TT and HPP samples were similar to each other with regard to flavor characteristics over the course of the shelf-life.

There was a general trend for the samples to be perceived as less *sour* and less *sweet* over time and for *strength of flavor* to diminish in response to increasing storage time. In contrast the *processed flavor* and *bitterness* of the samples became more pronounced as the shelf-life increased.

3.1.3. Aftertaste attributes

Mean panel scores for Navel orange juice aftertaste attributes are shown in Table 4. By week 1, the U 10 °C sample had the most intense *sour* and *bitter aftertaste* of all samples, but there was no significant difference between

Table 4
Sensory panel scores for Navel orange juice aftertaste attributes over 12 weeks storage

Attribute	Time	Juice samples						
	(Week)	U −20 °C[a]	U 4 °C	U 10 °C	TT 4 °C[b]	TT 10 °C	HPP 4 °C[c]	HPP 10 °C
Acid aftertaste	0	36.9	41.0	40.5	38.6	37.8	39.0	43.0
	1	29.2	28.4	33.7	31.7	27.6	28.8	39.3
	2	22.6	21.4		22.5	17.2	20.3	28.1
	4	30.1			20.0	20.2	23.3	33.9
	8	15.0			11.4		15.0	14.6
	12	16.2			11.5		11.6	12.8
Sour aftertaste	0	20.4	20.8	26.7	27.3	16.8	27.6	23.3
	1	20.2	24.4	39.5	29.3	25.9	28.4	24.0
	2	19.2	28.2		19.6	20.3	22.8	27.3
	4	33.1			27.8	24.6	25.7	37.3
	8	17.4			9.1		14.1	14.6
	12	16.2			12.6		12.9	14.2
Bitter aftertaste	0	19.0	24.6	31.8	29.3	17.0	28.8	30.3
	1	14.8	23.7	40.8	29.2	26.4	28.5	27.8
	2	20.6	27.2		21.5	19.0	27.5	29.4
	4	32.6			33.8	36.6	38.4	48.3
	8	25.8			21.8		22.0	22.5
	12	23.5			18.2		18.1	19.9
Duration of aftertaste	0	51.3	56.8	57.2	62.2	53.2	55.0	55.8
	1	49.4	45.6	51.4	49.2	45.1	50.6	53.7
	2	41.6	45.9		41.4	39.1	43.6	44.2
	4	62.9			48.8	52.9	55.4	62.3
	8	41.6			27.2		35.8	37.8
	12	45.2			42.1		43.5	45.3

[a]U=untreated juice; [b]TT=temperature treated juice; and [c]HPP=high pressure processed juice.

any of the samples with regard to *acid aftertaste* or *duration of aftertaste* at this point. The general trend across samples was for a decrease in *acid aftertaste*, *sour aftertaste*, *bitter aftertaste* and *duration of aftertaste* as the storage time increased.

Overall, the effect of storage on the sensory attributes of all the Navel orange juice treatments follows a general trend: namely a decrease in *sweet odor* and *sweet flavor*, *overall strength of orange odor* and *overall strength of orange flavor*, *sour flavor*, *acid aftertaste*, *sour aftertaste* and *duration of aftertaste*, alongside an increase in *aged odor*, *artificial odor*, *fermented odor*, and *processed flavor.* These sensory changes would suggest that microbiological fermentation was occurring during the course of the shelf-life trial, as was indeed the case (Bull et al., 2004). Additionally, *bitter flavor* and *bitter aftertaste* decreased in most samples as they aged but increased in the control sample over time.

3.2. Consumer acceptability

Mean consumer acceptance ratings for the Navel orange juice samples are shown in Table 5. Whilst the consumers initially liked most of the samples (as indicated by mean scores >5) the average scores for the Navel juices were not particularly high. This indicates that the samples were not well liked and consumers' comments indicated that the Navel juice was too acidic or too bitter for most consumers' liking. Scores <5 indicate that a sample was unacceptable to the consumers and it was concluded that any samples with such scores had reached the end of their shelf-life. Juice shelf-life as indicated by consumer acceptability is shown in Table 6.

At the baseline evaluation, there were significant ($p<0.05$) differences between the samples in terms of overall consumer acceptability. The U 10 °C and HPP 10 °C

Table 5
Consumer acceptability scores (mean) for Navel orange juice over 12 weeks storage

Time	Juice samples							
(Week)	U −20 °C[a]	U 4 °C	U 10 °C	TT 4 °C[b]	TT 10 °C	HPP 4 °C[c]	HPP 10 °C	Commercial juice
0	6.57	5.69	4.97	5.54	6.77	6.83	4.83	NT[d]
1	5.64	4.73	2.76	5.18	5.48	4.97	4.39	NT
2	4.76	4.94	NT	5.82	5.18	5.30	4.73	NT
4	4.40	NT	NT	5.07	5.50	4.93	5.07	NT
8	4.26	NT	NT	5.06	NT	4.94	4.26	NT
12	5.47	NT	NT	6.09	NT	5.56	4.44	6.27

1=dislike extremely; 5=neither like nor dislike; and 9=like extremely.
[a]U=untreated juice; [b]TT=temperature treated juice; [c]HPP=high pressure processed juice; and [d]NT=not tested.

Table 6
Shelf life of Australian Navel orange juice samples as indicated by consumer acceptability scores

Sample treatment	Shelf life (weeks)
U −20 °C[a]	≥12
TT 4 °C[b]	≥12
HPP 4 °C[c]	≥12
HPP 10 °C	4–12
TT 10 °C	4
U 4 °C	<1
U 10 °C	<1

[a]U=untreated juice; [b]TT=temperature treated juice; and [c]HPP=high pressure processed juice.

samples were liked significantly less than the U −20 °C (control), U 4 °C, TT 4 °C, TT 10 °C and HPP 4 °C samples. By week 1 of shelf-life, all samples were liked significantly less than at baseline. In particular, the untreated juice samples stored at 4 and 10 °C were unacceptable to consumers and the HPP 10 °C sample was also disliked slightly. The U −20 °C, HPP 4 °C and both TT samples were still acceptable to consumers, with the U −20 °C sample being liked significantly more than the HPP 4 °C and TT 10 °C samples.

After 2 weeks storage there were no significant differences between the samples for overall acceptability with all samples receiving a median score of 5.0 (equivalent to 'neither like nor dislike'). The results for week 4 showed that, surprisingly, the U −20 °C sample was disliked by the consumers and was the least liked sample at that time. The other samples (HPP and TT samples at both 4 and 10 °C) were still 'neither like nor disliked' by the consumers. By week 8, the mean scores for overall acceptability suggested that the U −20 °C and the HPP 10 °C samples were not liked, whilst the TT 4 °C and HPP 4 °C samples were 'neither liked nor disliked'.

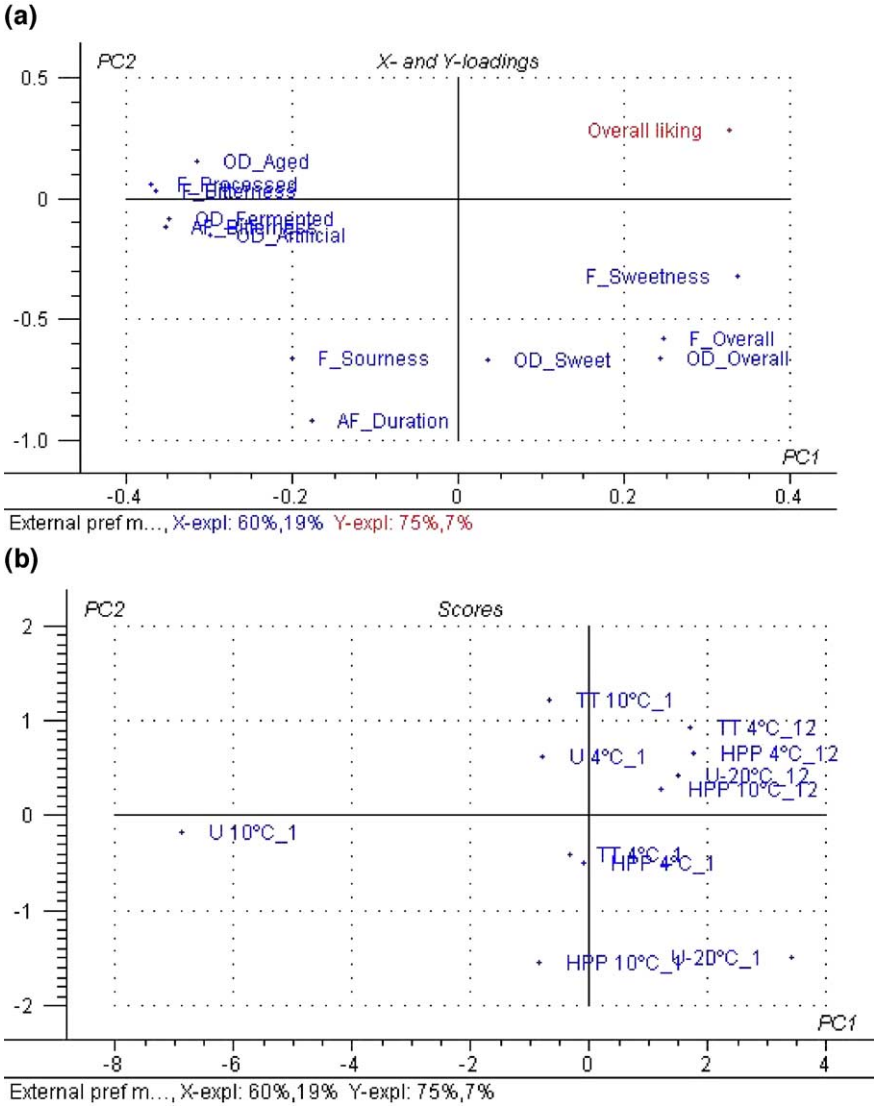

Fig. 2. External preference map of consumer acceptability for Navel orange juice samples over 12 weeks of storage: (a) attribute plot and (b) sample plot. The data from week 4 was not included in the external preference map analysis due to the deviation from the general trend over time for samples at this time point.

However, ANOVA of the acceptability data did not find any significant differences between the four samples, showing that consumers did not distinguish between these samples in terms of overall acceptability.

By the final week of testing (week 12 of shelf-life) the TT 10 °C sample was judged by the sensory scientist to be unacceptable for consumption. The results (median scores) from consumer analysis of the remaining samples showed that the HPP 4 °C, TT 4 °C and U −20 °C samples were all 'liked slightly' by the consumers whereas the HPP 10 °C was 'disliked slightly'. It was clear from the ANOVA that the consumers did not differentiate between the U −20 °C, HPP 4 °C and TT 4 °C samples, but these three samples were liked significantly more than the HPP 10 °C sample.

3.3. Effect of the sensory attributes of the Navel juice samples on consumer acceptability

Examining the contribution of each sensory attribute to the model on consumer preference (using the jack-knifing method) showed that attributes *color*, *acid aftertaste* and *sour aftertaste* did not have much influence on the PLSR1 model, so the analysis was re-run minus these attributes, improving the correlation between sensory attributes and consumer preference and also improving the predictive ability of the model.

The external preference map is shown in Fig. 2. It was found that 79% of the variation between the sensory characteristics of the orange juices could explain 82% of the variation in consumer preferences. The model had a correlation coefficient of 0.96 and a validation value (predictive ability) of 0.84.

In the external preference map (Fig. 2), the further an attribute is from the origin, the more that attribute contributes to consumer preference for the orange juice samples. The attribute plot (top plot in Fig. 2) shows that all the sensory attributes (once *color*, *acid aftertaste* and *sour aftertaste* were removed) were important determinants of consumer preference. The flavor attribute *sweetness* ('F_Sweetness'), *overall strength of orange odor* ('OD_Overall') and *overall strength of orange flavor* ('F_Overall') were aligned with overall liking, demonstrating that these attributes were important for consumer acceptability, as was *sweet odor* ('OD_Sweet').

Attributes negatively associated with consumer preference were those on the left-hand side of the attribute plot, associated with the negative pole of Principal Component 1 (the *X*-axis). These included *aged odor* ('OD_Aged'), *fermented odor* ('OD_Fermented') and *artificial odor* ('OD_Artificial'), *processed flavor* ('F_Processed') and *bitter flavor* ('F_Bitterness') and *bitter aftertaste* ('AF_Bitterness').

The sample plot (bottom plot of Fig. 2) shows that the preferred samples were the U −20 °C at week 1 and the TT 4 °C, HPP 4 °C, HPP 10 °C and U −20 °C samples, all at week 12. The only sample that was disliked was the U 10 °C sample at week 1, but it must be noted that samples were pulled from the trained panel and consumer analysis if microbiological tests deemed them to be unfit for consumption and samples were not included in the consumer sensory analysis if the sensory staff judged them to be too unpleasant.

3.4. Chemical analysis

Twenty-two volatile compounds have been identified as important in the flavor of orange juice (Shaw, 1991). These compounds are recorded in Table 7 together with their linear retention indexes (LRI), flavor threshold values (FTV) in water and concentration ranges in fresh and processed juice (all data taken from Shaw, 1991). Of these compounds sinensal and ethyl 2-methylbutanoate were not detected in any of our juice samples. As a consequence, our studies have concentrated on the remaining 20 compounds. The relative concentrations (RC) of these compounds in juice samples stored over a period of 12 weeks are reported in Tables 8–10. With the exception of the U −20 °C (control juice), the other six juices were stored at both 4 and 10 °C.

At week zero, the RC of the selected compounds was relatively consistent for all seven juices (Table 8). However,

Table 7
Volatile compounds important in the flavor of orange juice[a]

Compound	LRI[b]	FTV (water)[c] (μg/ml)	Concentration fresh (μg/ml)	Concentration processed (μg/ml)
Alcohols				
Ethanol	<600	53	380	260
(*E*)-2-hexenol	895	–	<0.01	–
(*Z*)-3-hexenol	887	0.07	<0.01–0.5	0.02
Linalool	1139	0.0038	0.15–2.34	0.6
α-terpineol	1257	0.3	0.09–1.1	0.1–0.8
Aldehydes				
Acetaldehyde	<600	0.022	3.0–7.0	1.2–3.3
(*E*)-2-pentenal	790	0.15	<0.01	0.0075
Octanal	1042	0.0005	<0.01–0.3	0.88
Nonanal	1146	0.0043	<0.01–0.04	0.09
Decanal	1251	0.0032	0.01–0.15	0.84
Citral	1322	0.041	0.05–0.3	0.48
Sinensal	ND[d]	0.038	–	0.11
Esters				
Ethyl acetate	<600	3.0	0.4	0.2
Ethyl propanoate	737	0.005	0.1	–
Methyl butanoate	749	0.059	0.1	0.16
Ethyl butanoate	828	0.00013	0.08–1.4	0.4
Ethyl 2-methylbutanoate	881	0.0001	<0.01–0.1	–
Ethyl 3-hydroxy hexanoate	1169	–	0.5–1.0	0.13
Terpene hydrocarbons				
α-limonene	1076	0.21	1.0–80	135–180
Myrcene	1023	0.042	0.05–2.0	2.6
α-pinene	976	1.0	0.02–0.09	0.38
Valencene	1591	–	0.04–0.2	1.05

[a]All data taken from Shaw (1991); [b]LRI=linear retention index, determined in the current study; [c]FTV=flavor threshold value, as reported by Shaw (1991); and [d]ND=not determined.

Table 8
Relative concentrations (μg/ml) of volatile flavor compounds in Navel orange juice samples at week zero and stored for 1 week

Compound	Juice samples													
	Week zero							Week 1						
	U −20 °C[a]	U 4 °C	U 10 °C	TT 4 °C[b]	TT 10 °C	HPP 4 ° C[c]	HPP 10 °C	U −20 °C	U 4 °C	U 10 °C	TT 4 °C	TT 10 °C	HPP 4 °C	HPP 10 °C
Alcohols														
Ethanol	10	8.7	8.7	7.6	7.6	11	11	10	3.2	2.9	7.5	8.7	10	8.7
(*E*)-2-hexenol	0.01	0.02	0.02	ND[d]	ND	ND	ND	ND	0.01	0.01	ND	ND	ND	ND
(*Z*)-3-hexenol	0.02	0.04	0.04	ND	ND	0.03	0.03	0.02	0.02	0.02	ND	ND	0.02	ND
Linalool	1.3	1.3	1.3	1.5	1.5	1.5	1.5	1.3	0.5	0.5	1.5	1.6	1.9	1.6
α-terpineol	0.3	0.2	0.2	0.2	0.2	0.2	0.2	0.1	0.3	0.09	0.2	0.2	0.2	0.2
Aldehydes														
Acetaldehyde	0.8	0.1	0.1	ND	ND	1.1	1.1	0.2	0.05	1.5	0.01	0.2	0.2	0.2
(*E*)-2-pentenal	0.01	0.01	0.01	<0.01	<0.01	<0.01	<0.01	<0.01	ND	ND	ND	ND	ND	ND
Octanal	0.9	0.8	0.7	1.3	1.3	1.0	1.0	0.9	0.03	ND	0.9	1.0	1.1	1.0
Nonanal	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	ND	ND	0.01	0.01	0.01	0.01
Decanal	0.07	0.05	0.05	0.1	0.1	0.06	0.06	0.05	0.01	<0.01	0.06	0.06	0.08	0.06
Citral	0.7	0.6	0.6	0.9	0.9	0.6	0.6	0.5	0.04	0.01	0.4	0.4	0.7	0.4
Esters														
Ethyl acetate	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.4	0.2	0.3	0.5	0.5	0.6	0.5
Ethyl propanoate	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	<0.01	ND	0.02	0.02	0.02	0.02
Methyl butanoate	0.05	0.05	0.05	0.07	0.07	0.05	0.05	0.04	0.01	0.01	0.05	0.08	0.07	0.08
Ethyl butanoate	3.4	3.8	3.8	4.9	4.9	3.9	3.9	2.5	0.5	0.2	3.7	5.2	5.0	5.2
Ethyl 3-hydroxy hexanoate	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.1	0.1	0.2	0.2	0.3	0.2	0.3
Terpene hydrocarbons														
α-limonene	73	47	47	68	68	65	65	75	12	13	62	60	75	60
Myrcene	0.4	0.3	0.3	0.4	0.4	0.4	0.4	0.3	0.02	0.03	0.3	0.3	0.4	0.3
α-pinene	0.02	0.01	0.1	0.01	0.01	0.01	0.01	0.01	ND	<0.01	0.01	0.01	0.01	0.01
Valencene	0.3	0.3	0.3	0.2	0.2	0.2	0.2	0.08	0.04	0.1	0.1	0.1	0.2	0.1

[a]U=untreated juice; [b]TT=temperature treated juice; [c]HPP=high temperature processed juice; and [d]ND=not detected.

some differences were apparent, (*E*)-2-hexenol, (*Z*)-3-hexenol and acetaldehyde were not detected in the two TT juices and acetaldehyde and limonene were present in much lower concentrations in the U 4 °C and U 10 °C juices. (*E*)-2-hexenol was also not detected in the two HPP juices. Even so, for the majority of compounds the concentrations found in the unprocessed and processed juices lie within the ranges reported by Shaw (1991) for fresh and processed juice. A major exception was ethanol. The concentrations of this compound were all about 3% of reported values.

To establish the possible role that individual compounds could have in the flavor of the juices, the RC of each compound has been compared with the corresponding FTV reported in Table 7. We have assumed that these values, determined in water, will be about the same as those in orange juice. In the U −20 °C (control) sample at week zero, 11 compounds, linalool, α-terpineol, acetaldehyde, octanal, nonanal, decanal, citral, ethyl propanoate, ethyl butanoate, limonene and myrcene (Table 8), had RC equal to or greater than their reported FTV (Table 7). As four of these compounds, ethyl butanoate, octanal, limonene and linalool, greatly exceeded their FTV in all juices, they could be expected to have an impact on the perceived flavors of these products.

After storage for 1 week, the RC of the selected compounds remained comparable with the control, TT and HPP juices. However, in the untreated juices (U 4 °C and U 10 °C) notable decreases in concentrations were observed for linalool, octanal, citral, ethyl butanoate, limonene and myrcene (Table 8). In the U −20 °C, TT and HPP juices, between 10 and 11 compounds exceeded their FTV but in the U 10 °C juice only four compounds, linalool, acetaldehyde, ethyl butanoate and limonene, exceeded these values.

By week 2 the concentrations for most compounds were comparable across U −20 °C, TT and HPP 4 °C juices (Table 9). The FTV of between 10 and 11 compounds were exceeded in these juices and these were the same compounds as those identified in juices at week zero (Table 8). As for the HPP 10 °C juice, the RC of most compounds were appreciably less than those observed in the U −20 °C and only seven compounds in this juice exceeded their FTV. The RC of most compounds in the U 4 °C juice at week 2 were similar to those observed at week 1 (Table 8). By comparison the U 10 °C juice had elevated levels of ethanol, acetaldehyde, ethyl acetate and limonene. The first three of these compounds were typical products of fermentation but the large increase in limonene

Table 9
Relative concentrations (μg/ml) of volatile flavor compounds in Navel orange juice samples stored for 2 and 4 weeks

Compound	Juice samples												
	Week 2							Week 4					
	U −20 °C[a]	U 4 °C	U 10 °C	TT 4 °C[b]	TT 10 °C	HPP 4 °C[c]	HPP 10 °C	U −20 °C	U 4 °C	TT 4 °C	TT 10 °C	HPP 4 °C	HPP 10 °C
Alcohols													
Ethanol	14	6.5	26	9.5	10	13	4.1	13	21	16	10	2.6	4.5
(*E*)-2-hexenol	ND[d]	0.01	ND	ND	ND	ND	ND	ND	0.01	ND	ND	ND	ND
(*Z*)-3-hexenol	0.01	0.03	0.02	ND	ND	0.02	0.02	0.01	0.03	ND	ND	0.02	0.01
Linalool	1.9	0.6	1.0	1.6	1.7	2.1	0.6	1.7	1.4	1.2	1.5	2.0	1.7
α-terpineol	0.2	0.4	0.1	0.2	0.2	0.2	0.1	0.2	0.3	0.2	0.3	0.3	0.8
Aldehydes													
Acetaldehyde	0.3	0.1	7.6	0.06	0.2	0.3	0.09	0.3	2.9	0.02	1.1	0.9	0.7
(*E*)-2-pentenal	ND	ND	ND	ND	ND	ND	ND	<0.01	ND	ND	ND	ND	ND
Octanal	1.3	0.01	ND	0.9	1.0	0.9	0.2	1.1	0.01	0.6	0.03	0.9	0.6
Nonanal	0.01	ND	ND	0.01	0.01	0.01	ND	0.01	<0.01	0.01	ND	0.01	0.01
Decanal	0.09	0.01	0.01	0.06	0.05	0.06	0.01	0.1	0.01	0.05	0.01	0.05	0.03
Citral	0.9	0.01	ND	0.2	0.2	0.3	0.03	1.0	0.02	0.1	ND	0.2	0.08
Esters													
Ethyl acetate	0.5	0.1	7.6	0.6	0.7	0.6	0.2	0.4	0.1	0.3	1.2	ND	ND
Ethyl propanoate	0.02	<0.01	ND	0.02	0.02	0.02	0.01	0.02	ND	0.01	ND	ND	ND
Methyl butanoate	0.05	0.01	ND	0.06	0.09	0.06	0.02	0.04	0.01	0.04	0.01	ND	<0.01
Ethyl butanoate	2.9	0.3	0.2	3.9	5.8	3.9	1.4	2.6	0.4	2.2	2.6	2.3	2.4
Ethyl 3-hydroxy hexanoate	0.08	0.1	0.2	0.2	0.3	0.2	0.1	0.1	0.2	0.1	0.3	0.2	0.2
Terpene hydrocarbons													
α-limonene	74	17	150	80	70	81	20	63	28	41	37	45	20
Myrcene	0.4	0.09	0.9	0.5	0.4	0.5	0.04	0.3	0.2	0.2	0.3	0.2	0.1
α-pinene	0.01	<0.01	0.03	0.01	0.02	0.01	<0.01	0.01	<0.01	0.01	0.01	0.01	<0.01
Valencene	0.08	0.2	0.7	0.09	0.2	0.5	0.1	0.2	0.01	0.05	0.1	0.3	0.1

[a]U=untreated juice; [b]TT=temperature treated juice; [c]HPP=high temperature processed juice; and [d]ND=not detected.

is difficult to explain. As the remaining three samples of this juice all showed visible signs of fermentation (gas production) no further samples of the U10 °C juice were analyzed. After 4 weeks storage, the RC of most of the selected compounds in the U −20 °C juice (Table 9) were similar to those found at week zero (Table 8). By comparison, the four processed juices showed reduction in concentrations of some compounds, particularly citral and limonene. Even so, 10 compounds exceeded their FTV in three of the processed juices. The exception was the TT 10 °C juice where the FTV of only eight compounds were exceeded. However, the U 4 °C had lower concentrations of five compounds, citral, limonene, octanal, ethyl butanoate and valencene. This juice also had elevated levels of ethanol and acetaldehyde (Table 9).

By week 8 and week 12 (Table 10) the composition of the different juices appeared to have stabilized. However, the RC of all compounds in both the untreated and processed juices had decreased when compared with those found at week zero (Table 8) and for the majority of compounds the concentrations were comparable across all six juices. Compounds not following this trend were citral, only found in the U −20 °C juices, and octanal and ethyl butanoate that were present in much lower concentrations in the U 4 °C juice (Table 10). By week 8 and week 12, 10 compounds were present in the U −20 °C juices in concentrations equal to or greater than their FTV, whereas in the two HPP and TT 10 °C juices, nine compounds were equal to or exceeded their FTV. By comparison, the TT 4 °C juices had eight compounds at both week 8 and week 12 that exceeded their FTV, whereas the U 4 °C juices had seven and six compounds, respectively, that were equal to or exceeded these values. Based on the relative composition of these six juices at week 8 and week 12 it might be expected that the U −20 °C juices would have marginally superior flavors to those of the HPP and TT 10 °C juices. Particularly, the compound absent from the processed juices was citral, a compound recognized for its characteristic orange-like aroma (Shaw, 1991). However the flavors of the TT 4 °C juices could be very similar to those of the TT 10 °C, HPP 4 °C and HPP 10 °C juices as the only difference between these juices were lower concentrations of acetaldehyde in the TT 4 °C juices. By comparison, the flavors of the U 4 °C juices could be expected to be inferior to the control and processed juices. These U 4 °C juices, in addition to the absence of the important flavor compounds, nonanal, citral and ethyl propanoate, also had relatively lower concentrations of ethyl butanoate than the other juices (Table 10).

Table 10
Relative concentrations (μg/ml) of volatile flavor compounds in Navel orange juice samples stored for 8 and 12 weeks

Compound	Juice samples											
	Week 8						Week 12					
	U −20 °C[a]	U 4 °C	TT 4 °C[b]	TT 10 °C	HPP 4 °C[c]	HPP 10 °C	U −20 °C	U 4 °C	TT 4 °C	TT 10 °C	HPP 4 °C	HPP 10 °C
Alcohols												
Ethanol	0.8	1.1	1.1	1.4	0.4	1.1	1.1	2.0	2.7	0.2	0.9	1.0
(*E*)-2-hexenol	ND[d]	<0.01	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
(*Z*)-3-hexenol	0.01	0.04	ND	ND	0.01	0.01	0.01	0.04	ND	ND	0.01	0.01
Linalool	0.5	0.6	0.6	0.7	0.6	0.7	0.8	0.6	0.7	0.7	0.7	0.7
α-terpineol	0.1	0.1	0.1	0.2	0.09	0.1	0.1	0.2	0.1	0.2	0.1	0.2
Aldehydes												
Acetaldehyde	0.1	0.6	0.01	0.2	0.1	0.1	0.2	ND	<0.01	0.02	0.03	0.02
(*E*)-2-pentenal	<0.01	ND	ND	ND	ND	ND	<0.01	ND	ND	ND	ND	ND
Octanal	0.3	0.02	0.3	0.2	0.3	0.3	0.3	0.02	0.2	0.1	0.3	0.2
Nonanal	<0.01	ND	<0.01	<0.01	<0.01	<0.01	<0.01	ND	<0.01	<0.01	<0.01	<0.01
Decanal	0.01	<0.01	0.01	0.01	<0.01	<0.01	0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Citral	0.04	ND	ND	ND	ND	ND	0.04	ND	ND	ND	ND	ND
Esters												
Ethyl acetate	0.1	0.1	0.1	0.1	ND	0.06	0.1	0.1	0.2	0.1	0.1	0.06
Ethyl propanoate	<0.01	ND	<0.01	0.01	<0.01	<0.01	0.01	ND	0.01	<0.01	0.01	<0.01
Methyl butanoate	0.01	<0.01	0.01	0.02	0.02	0.02	0.02	<0.01	0.04	0.02	0.02	0.01
Ethyl butanoate	1.3	0.2	1.6	1.9	2.0	2.0	1.6	0.1	3.1	1.5	1.7	1.4
Ethyl 3-hydroxy hexanoate	0.05	<0.01	ND	0.2	0.1	0.2	0.05	0.1	0.09	0.1	0.1	0.1
Terpene hydrocarbons												
α-limonene	19	24	19	24	21	23	28	17	18	15	20	16
Myrcene	0.1	0.2	0.1	0.2	0.1	0.2	0.2	0.1	0.2	0.1	0.2	0.1
α-pinene	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Valencene	0.01	0.07	0.02	0.04	0.03	0.04	0.04	0.02	0.02	0.01	0.03	0.02

[a]U=untreated juice; [b]TT=temperature treated juice; [c]HPP=high tempearature processed juice; and [d]ND=not detected.

Considerable reductions in the RC of most compounds were observed in all juices held for 4 weeks at 4 and 10 °C (Table 9) compared with those of the corresponding juices at week zero (Table 8). Those compounds showing the greatest reductions in concentrations were octanal, citral, ethyl butanoate and limonene. However, by week 8, all juices, including U −20 °C, showed comparable reductions in concentrations for most compounds (Table 10) compared with those at week zero (Table 8). In the U −20 °C juice, the concentration of limonene had decreased from 73 to 19 μg/ml, ethyl butanoate from 3.4 to 1.3 μg/ml, linalool from 1.3 to 0.5 μg/ml, octanal 0.9 to 0.3 μg/ml and citral from 0.7 to 0.04 μg/ml (Tables 8 and 10). Similar reductions in concentrations have been reported by Moshonas and Shaw (2000) for ethyl butanoate, octanal and citral (geranial) in orange juices stored in laminated cartons for 7 weeks at 2 °C. The concentrations of water-soluble compounds were found to be reduced to 30% of their original value and those of oil-soluble compounds to 70%, after storage for 5 weeks. These authors also found that for some compounds reduction in concentrations of up to 50% occurred when the juice was stored at −18 °C. Further reductions were not observed over the next 4 weeks, when the study was terminated. The major cause of these losses was absorption by the polymeric liner of the packaging material (Moshonas & Shaw, 2000). In our studies we found the concentrations of most compounds stabilized at week 8 (Table 10).

The final concentrations of these compounds at week 12 varied between 6% and 38% of the original levels. In the current study, polyethylene terephthalate (PET) bottles were selected for their suitability for high pressure processing and reported low absorption of most volatile orange juice components (Nielsen, 1994). In studies of the interaction between PET and aroma compounds it has been shown that only 1–2% of the total content of myrcene and limonene were absorbed in refillable PET bottles and that the absorption rate was temperature dependent (Nielsen, 1994). More recently, Sheung, Min and Sastry (2004) showed that PET packaging reduced the absorption of D-limonene and α-pinene from orange juice as compared to other packaging materials studied. However, analyses of PET bottles used for the storage of control samples at −20 °C for more than 12 weeks showed considerable absorption of the majority of compounds covered by our survey. Furthermore, although no attempt was made to quantify the levels of compounds absorbed by the PET, the total counts recorded by the GC–MS indicated that the quantities of limonene and

Table 11
Partial least squares regression of individual sensory attributes (*Y* variables) as a function of volatile compounds (alcohols and aldehydes; *X* variables) found in Navel orange juice

Attribute	Correlation[a]				Volatile compound β-coefficient (alcohols and aldehydes)										
	Calibration	Validation	Factors	RMSEP	Ethanol	(*E*)-2-hexenol	(*Z*)-3-hexenol	Linalool	α-terpineol	Acetaldehyde	(*E*)-2-pentenal	Octanal	Nonanal	Decanal	Citral
Odor															
Sweet	0.88	0.84	2	10.27	–	0.14*	0.10	–	0.06	–	0.23*	0.11*	–	0.11*	0.16*
Aged	0.50	0.05	2	9.07	–	0.03	−0.05	–	0.15	−0.06	−0.21	–	−0.06	–	−0.09
Artificial	0.59	0.37	2	6.45	–	–	−1.09	0.55	0.60	–	−2.09*	–	–	–	−0.85
Fermented	0.35	0.00	2	5.89	0.11	0.32	0.14	0.22*	0.41	−0.08	0.05	0.18	−0.03	0.21*	0.16
Strength	0.78	0.66	2	12.96	–	–	–	1.47*	0.98*	−0.51	1.18*	1.64*	1.23*	1.52*	1.50*
Flavor															
Sour	0.85	0.75	2	9.65	–	0.21*	0.08	0.05*	0.07	–	0.13*	0.09*	–	0.09*	0.12*
Sweet	0.76	0.70	2	12.47	–	0.11	–	−0.01	0.06	−0.09	0.25*	0.07*	0.15	0.04	0.07
Processed	0.36	0.35	2	12.39	−0.03	0.03	−0.04	0.03	0.04	−0.05	–	0.04	0.03	0.03	–
Bitterness	0.39	0.11	2	11.22	0.01	0.02	–	0.05*	0.03	−0.03	–	0.04*	0.03	0.04	0.03
Strength	0.81	0.73	2	13.28	–	0.04	–	0.06*	0.09	−0.08	0.14*	0.10*	0.08*	0.09*	0.10
Aftertaste															
Acid	0.86	0.79	2	8.62	–	0.18*	0.13*	–	0.09	–	0.18*	0.10*	–	0.10*	0.15*
Sour	0.59	0.43	1	9.52	0.03	0.03	–	0.06*	0.05*	−0.02	0.03*	0.06*	0.03	0.03*	0.06*
Bitter	0.53	0.36	1	10.43	–	0.03	–	0.06*	0.04	−0.03	0.04*	0.06*	0.05	0.06*	0.05*
Duration	0.75	0.60	2	15.96	–	0.10	0.05	0.05*	0.07	−0.08	0.13*	0.08*	0.08	0.07*	0.08*

[a]Factors=number of PLS factors in the correlation model; RMSEP=root mean square error of prediction; and *=significant variable ($p < 0.05$) as per the method of Martens uncertainty test.

myrcene present greatly exceeded the 1–2% reported by Nielsen (1994). Even so, it is unlikely that absorption alone can account for all of the losses observed in our investigation. Chemical reactions such as oxidation, hydrolysis and acid-catalyzed reactions must be considered as alternative causes for the reduction in aroma compounds in our untreated and processed juices (Kutty, Braddock & Sadler, 1994).

3.5. Correlation of flavor attributes with volatile compounds

PLS regression was used to compare combinations of volatile compounds that correlated with variation in the sensory response (individual flavor attributes) to the orange juice consumption. The fit (calibration), predictive ability (validation) and predictive error (RMSEP) of each PLS-type 1 model are shown in Tables 11 and 12. The number of PLS factors taken into consideration and the contribution of each volatile (β-coefficient) to the PLS regression models are also shown in these tables.

Significant relationships were found between combinations of volatiles and individual sensory attributes of the Navel orange juices (Tables 11 and 12). For example, *sweet odor* (RMSEP=10.27) was correlated with an increase in (*E*)-2-hexenol, (*E*)-2-pentenal, octanal, decanal, citral, ethyl propanoate, methyl butanoate and ethyl butanoate. Tables 11 and 12 also show the important contribution to Navel orange juice odor, flavor and aftertaste made by the volatile compounds (*E*)-2-pentenal, octanal, decanal, citral, ethyl propanoate, methyl butanoate and ethyl butanoate, with an increase in these volatile compounds being significantly correlated with the majority of sensory attributes.

3.6. Acceptability of stored orange juice

The shelf-life of the seven samples of untreated and processed orange juices is recorded in Table 6. Of these juices only three, U −20 °C, HPP 4 °C and TT 4 °C, were still acceptable to consumers after 12 weeks storage. Tasting of the other juices, with the exception of HPP 10 °C, had ceased by week 8 for microbiological safety reasons. By comparison, analysis of the volatile compounds was continued for the full 12 weeks for six of the juices. The exception was U 10 °C juice that was discarded after 2 weeks storage.

At the commencement of this study it was assumed that the volatile content of the U −20 °C juices would remain reasonably constant and that any variation in the concentrations of the selected compounds would only be observed in the processed juices stored at 4 and 10 °C. The analytical data initially appeared to justify these assumptions when at week 4 the concentrations of several compounds had decreased appreciably in the juices stored at 4 and 10 °C compared with those of the U −20 °C juice (Table 9). However, these decreases were not reflected in the sensory data as the *strength of orange odor* and *strength of orange flavor* for the control and processed juices all received similar scores (Fig. 1 and Table 3). The sensory data can be explained by the fact that although the concentrations of several compounds in the processed juices were less than in

Table 12
Partial least squares regression of individual sensory attributes (*Y* variables) as a function of volatile compounds (esters and terpene hydrocarbons; *X* variables) found in Navel orange juice

Attribute	Correlation[a]				Volatile compound β-coefficient (esters and terpene hydrocarbons)								
	Calibration	Validation	Factors	RMSEP	Ethyl acetate	Ethyl propanoate	Methyl butanoate	Ethyl butanoate	Ethyl-3-hydroxy hexanoate	α-limonene	Myrcene	α-pinene	Valencene
Odor													
Sweet	0.88	0.84	2	10.27	−0.05	0.14*	0.07*	0.09*	–	–	–	0.05	–
Aged	0.50	0.05	2	9.07	−0.07	0.02	–	–	0.07	−0.06	−0.10	−0.09	−0.07
Artificial	0.59	0.41	2	6.93	–	0.51	0.67	–	1.77	–	−1.01	−0.76	−1.00
Fermented	0.35	0.02	2	5.89	−0.18	0.25	0.24	0.19	0.40	–	−0.07	−0.15	0.04
Strength	0.75	0.69	2	12.41	−0.42	1.85*	1.49*	1.53*	1.01*	–	–	–	–
Flavor													
Sour	0.85	0.74	2	10.59	–	0.15*	0.10*	0.10*	0.12	−0.06	−0.11	0.09	–
Sweet	0.78	0.70	2	12.48	−0.11	0.16*	0.03	0.08*	–	–	–	–	–
Processed	0.36	0.36	2	12.39	−0.06	0.05	0.05*	0.05	0.08	−0.03	−0.05	−0.06	−0.04
Bitterness	0.39	0.11	2	11.22	−0.03	0.05	0.05*	0.05*	0.06*	–	–	−0.01	–
Strength	0.81	0.73	2	13.33	−0.09	0.16*	0.07*	0.08*	–	–	−0.03	–	–
Aftertaste													
Acid	0.86	0.79	2	9.26	−0.08	0.14*	0.07*	0.08*	0.10	–	−0.06	–	–
Sour	0.59	0.45	1	10.59	−0.02	0.07*	0.07*	0.06*	0.06*	0.03	–	–	0.02
Bitter	0.53	0.36	1	11.43	−0.03	0.08*	0.06*	0.06*	0.06*	–	–	0.02	–
Duration	0.75	0.60	2	16.26	−0.10	0.14*	0.07	0.08*	0.06	−0.03	−0.05	–	–

[a]Factors=number of PLS factors in the correlation model; RMSEP=root mean square error of prediction; and *=significant variable ($p<0.05$) as per method of Martens uncertainty test.

the U −20 °C juices (Table 9) their concentrations still exceeded the FTV for these compounds (Table 7). By comparison, the sensory data (Fig. 1 and Table 3) indicated that the TT 10 °C juice had a stronger *aged odor* (not significantly correlated by PLSR with any volatile compounds; Tables 11 and 12), *artificial odor* (significantly correlated with (*E*)-2-pentenal) and *fermented odor* (significantly correlated with linalool and decanal) than the other juices and that it also had a stronger *processed flavor* (significantly correlated with ethyl propanoate). However, the appearance of these off-odors and off-flavors does not appear to have any relationship with the presence or absence of the 20 compounds chosen for analysis.

The assumption that the volatile content of the U −20 °C juice would remain constant over 12 weeks storage was shown to be incorrect by the analysis of samples stored for 8 and 12 weeks (Table 10). In the U −20 °C juices the concentrations of most compounds had decreased so that they were now comparable with those juices stored at 4 and 10 °C. The only difference between the three processed juices still suitable for sensory analysis (TT 4 °C, HPP 4 °C and HPP 10 °C) and the U −20 °C juices was the absence of citral from all but the U −20 °C juices (Table 10). PLSR found the presence of citral to be significantly correlated with *sweet odor*, *overall strength of orange odor*, *sour flavor*, *overall strength of orange flavor*, and all aftertaste attributes (Table 11). These would all seem to be important attributes for the characteristic sensory qualities of orange juice, suggesting that the processed juices were lacking in these fresh juice characteristics by weeks 8–12. However, in agreement with the analytical data, the results from the sensory analyses (Fig. 1 and Table 3) showed no significant differences in scores for the odor and flavor attributes of the four juices. Accordingly, the odor and flavor of the three processed juices TT 4 °C, HPP 4 °C and HPP 10 °C would appear to be comparable with those of the control juices stored at −20 °C.

With regard to the consumer overall acceptability of the juices (Table 5) at week 8, the TT 4 °C and HPP 4 °C juices received a higher rating than the U −20 °C and HPP 10 °C juices, whereas at week 12, the TT 4 °C juice received a higher rating than the U −20 °C and HPP 4 °C juices with the HPP 10 °C juice receiving the lowest rating. These results would suggest that the consumer panel used sensations other than odor and flavor to rate these juices.

4. Conclusion

In the present study, Navel orange juice processed by HPP was shown to be stable for up to 12 weeks when stored at either 4 or 10 °C; by sensory analysis these juices were shown to have odor and flavor profiles comparable with those of a control juice stored at −20 °C and a TT juice stored at 4 °C. In addition, the three processed juices had marginally superior aftertaste attributes than the U −20 °C juice. However, according to consumer overall acceptability the HPP 10 °C juice was inferior to the U −20 °C, TT 4 °C and HPP 4 °C juices. Analysis by GC–MS of the four juices after 8 and 12 weeks storage showed that the volatile contents of these juices, as assessed by the presence or absence of 20 key aroma compounds, were very comparable. These findings would suggest that HPP of orange juice could produce a product acceptable to most consumers even after storage for 12 weeks at temperatures up to 10 °C.

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