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Producing ice cream using a substantial amount of juice from kiwifruit with green, gold or red flesh

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ABSTRACT

Ice cream prepared using a substantial amount of juice from kiwifruit with green, gold or red flesh may have consumer appeal, through the combination of kiwifruit's unique color, natural flavor and health-promoting constituents. The aqueous fractions from purees of kiwifruit with green, gold and red flesh (AFKWs) were added at 49% v/v to a basic low-fat ice cream mix that contained no commercial flavoring and coloring agents. The resultant ice creams were subjected to comparative product evaluation (e.g. overrun, melting behavior and rheological properties) and chemical analyses of bioactives (e.g. total extractable polyphenol content (TEPC), vitamin C, antioxidant capacity, polyphenol (PP) and carotenoid composition). Results revealed that both the pH pre-adjustment and pre-heating of the AFKW played critical roles in ice cream making. The ice creams retained the PP and vitamin C contents as well as natural color flavor of the kiwifruit used. The rheological properties of all ice creams showed non-Newtonian flow behavior, and the storage modulus G' decreased in the same pattern following the order of green>gold>red. The melting rate, overrun and vitamin C content of the ice cream with green AFKW were the fastest, lowest and least, respectively. The TEPC and antioxidant capacity in the ice cream with red AFKW were the highest. The amounts of PPs and vitamin C were encouragingly high. Health beneficial compounds, dimethyl-caffeic acid hexoside, caffeic acid derivatives, protocatechuic acid, syringic acid, salicylic acid/*o*-coumaric acid, lutein and β -carotene, were detected in the final products. Thus, there are commercial possibilities for using AFKW which should be further evaluated.

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1. Introduction

The market for foods that provide nutritional function and new eating experiences for consumers has grown rapidly in recent years (de Graaf, 2007; Tuorila, 2007; Van Kleef, van Trijp, Luning, & Jongen, 2002; Williams, Stewart-Knox, & Rowland, 2004). Ice cream is one of the most consumed dairy products in the world (Gorski, 1997; Hoyer, 1997) but the ice cream available commercially is generally poor in natural antioxidants like vitamin C, colors and polyphenols (PPs). Thus, it is of interest to explore the possibility of improving the nutritional attributes of ice cream using ingredients with health benefits, focusing on natural antioxidants, natural colorants, vitamins, low fat and freedom from synthetic additives in light of consumer expectations (El-Nagar, Clowes, Tudoric, Kuri, & Brennan, 2002; Gidley, 2004; Starling, 2005; Van Kleef et al., 2002).

Kiwifruit (*Actinidia* sp.) has many appealing consumer traits including flavor, color and nutritional content, especially vitamin C

(Ahmet, Sezai, & Nihat, 2007; Nishiyama, 2007). Consumption of kiwifruit offers health benefits including alleviating constipation and improving stool transit time and/or bulking (Chan, Leung, Tong, & Wong, 2007; Duttaroy & Jørgensen, 2004; Ferguson, Philpott, & Karunasinghe, 2004; Philpott, Mackay, Ferguson, Forbes, & Skinner, 2007; Rush, Patel, Plank, & Ferguson, 2002), because of constituents such as vitamin C, PPs, carotenoids and fiber polysaccharides (Dawes & Keene, 1999; Deters, Schröder, & Hensel, 2005; Lesperance, 2009). Dietary intake of vitamin C is essential for humans because humans cannot synthesize vitamin C (Davey et al., 2000; Ferraroni et al., 1994; Minnuni, Wolleb, Mueller, Pfeifer, & Aeschbacher, 1992; Rababah, Ereife, & Howard, 2005). A single kiwifruit contains the minimum daily requirement for vitamin C (Ferguson, 1991), suggesting that kiwifruit-derived products could be an excellent source of this essential vitamin. Kiwifruit flesh can have different colors derived from pigments such as chlorophyll, carotenoids and anthocyanins (Cano, 1991; Comeskey, Montefiori, Edwards, & McGhie, 2009; McGhie & Ainge, 2002; Nishiyama, Fukudo, & Oota, 2005; Possingham, Coote, & Hawker, 1980). Thus, kiwifruit may be suitable for the development of a new ice cream product with a range of natural colors and enhanced nutritional content.

Ice cream preparation typically involves a mix-making and a freezing stage, with important processing steps being blending, pasteurization, homogenization, cooling, flavoring and coloring,

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freezing, packaging, hardening and frozen storage (Schmidt, 2004). Ice cream is made from milk ingredients (milk fat and milk solids-not-fat), sugar, water, and other optional ingredients such as flavorings, colors, stabilizers, and emulsifiers (Schmidt, 2004). The ice cream flavor and color is normally generated through adding a small amount (i.e. approximately 0.3% ice cream mix) of liquid flavors and colors after aging and before freezing (Schmidt, 2004). Homogenization can reduce the particle size and aggregation of fat globules at low temperature, compensate the function of stabilizer, enhance ice cream viscosity but elevate the oxidation of milk fat and instability of proteins (Bodyfelt, Tobias, & Trout, 1988; Goff, 1999; Spreer, 1998; Sweetsur & Muir, 1983). The pressure of homogenization influences the viscosity but not the melting resistance (Thomsen & Holstborg, 1998). During aging, it is essential that the adsorbed protein is partially desorbed from the fat globule surface. Overrun and melting property are important variables for evaluating an ice cream product and associated processing (Arbuckle, 1986).

The use of juice from kiwifruit for ice cream production may eliminate the need to add commercially available flavoring and coloring agents, but also presents technical challenges that are associated with the nature of fruit juice. When fruit juice is mixed with milk protein, various interactions are possible. Interactions among PPs and proteins may lead to the formation of PP–protein complexes (Haslam, 1998; Perez-Jimenez & Saura-Calixto, 2006; Rawel, Kroll, & Hohl, 2001; Rohm, Rawel, & Kroll, 2004). Such complexation may result in effects such as milk stabilization on heating (O'Connell, Fox, Tan-Kintia, & Fox, 1998) and synergy in antioxidant capacity (Skrede, Larsen, Aaby, Jorgensen, & Birkeland, 2004). The added fruit juice could change the pH of the ice cream mix, which may lead to protein aggregation and peptide precipitation if the pH approaches the isoelectric point (pI) (Amundson, Watanawanichakorn, & Hill, 1982; Pearce, 1983), and induce hydrophobic interactions (Blondelle, Bittner, & Houghten, 1992; Bull & Breese, 1970; Léonil et al., 1994). Ice cream color may change with type of kiwifruit when various kiwifruit juices are added to milk. The naturally occurring pigments in kiwifruit (chlorophyll, carotenoids and anthocyanins) are generally unstable, but may be more attractive to consumers than synthetic coloring agents. The green pigments (chlorophyll a and b) tend to lose the Mg^{2+} ion to form brown pheophytin under acidic conditions (Cassano, Figoli, Tagarelli, Sindona, & Drioli, 2006; Johnson & Steele, 1995; Lodge & Perera, 1995). Carotenoids are particularly susceptible to heat destruction and oxidation, because of their highly unsaturated chemical structure (Stefanovich & Karel, 1982), while anthocyanins are degradable at high temperatures, and undergo structural transformations with the change in pH, forming interconvertible forms of red flavylum cation and blue quinoidal base (Kosir, Lapornik, Andersen, Vrhovsek, & Kidric, 2004). Thus processing conditions need to be carefully managed if these natural colorants are to be preserved in processed products.

This study aimed to examine the feasibility of producing a new type of ice cream product through incorporating a substantial amount of AFKW in the absence of any added flavoring agent, and provide insights into the chemical and physical properties of this frozen dessert format.

2. Materials and methods

2.1. Materials and chemicals

Pams® instant skim milk powder, Chelsea® white sugar, whole milk (Anchor®), green and gold kiwifruits were purchased from the Foodtown supermarket, St Lukes, Auckland. Red kiwifruit were obtained from Plant & Food Research, New Zealand. Soya lecithin (Yelkin, liquid) was obtained from Archer Daniels Midland Co., Decatur, IL, USA. Gelatin was obtained from Gelita NZ Ltd, Christchurch, New Zealand.

Methanol, *n*-hexane and formic acid were purchased from Ajax Finechem (Auckland, New Zealand). Acetone was from Burdick & Jackson (Muskegon, MI, USA). Folin–Ciocalteu phenol reagent, catechin, epicatechin, phlorizin, phloretin, quercetin, rutin, and *p*-coumaric, chlorogenic and caffeic acids were purchased from Sigma-Aldrich (St. Louis, MO, USA). Cyanidin 3-*o*- β -glucopyranoside chloride was sourced from Polyphenols Laboratories AS (Hanaven, Sandnes, Norway). Milli-Q Plus water was used for all the reagent preparation.

2.2. Ice cream preparation

Green, gold and red kiwifruits were firstly washed with water to remove any external contaminants. Both ends of each fruit were cut off and the skin was removed with a sterilized stainless peeler (APS001; Andefia, Zhongshan, China). Kiwifruits were pureed using a Juice Fountain Juicer (Model BJE 200C; Breville, Sydney, New South Wales, Australia), centrifuged at a speed of 5000 $\times$ g for 25 min at 4 °C using a Sorvall Instrument RC5C (DuPont, Michigan, WI, USA) and a Fibrelite F10s rotor (Piramoon Technologies Inc, Santa Clara, CA, USA), and then filtered through a sterilized mesh cloth to separate the AFKW from particles.

Ice cream was prepared using modified standard methods (Marshall & Arbuckle, 1996; Schmidt, 2004). The gelatin (4.54 g) was dissolved in water (30 mL) by gentle stirring over a boiling-water bath. Skim milk powder (135 g) was first mixed with sugar (61 g) to generate a “dry mix”. AFKWs (500 mL) were subjected to pre-treatment which included adjustment of pH to 4.7–4.9 using baking soda (Edmonds, from Goodmans Fielder Ltd., Auckland, New Zealand) and heating at 55 °C for 1 min, and then mixed with whole milk (500 mL) to generate the “liquid mix”. The “liquid mix” was sealed, covered with tin foil, and heated to 30–40 °C in a water bath. The “dry mix” was added slowly to the “liquid mix” with gentle stirring, and gelatin solution and liquid lecithin ingredient (5.54 g) were then added. The obtained mixture was blended using a mixer (L5T, Silverstone Machines Inc., East Longmeadow, MA, USA; emulsifying screen, 3000 rpm, 1-min plus 2-min burst), pasteurized in a double boiler at 80 °C for 30 s, homogenized using an Avestin EmulsiFlex C3 homogenizer (Ottawa, Canada) at 2000/500 psi, cooled to 4 °C, aged in the fridge at 4 °C for 20 h, frozen and whipped in the ice cream maker (Gelato di Amore M916, Artisan Gelato Systems, Bologna, Italy) at low speed for 23 min. The ice cream was collected at an exit temperature of -5.5 °C, placed in a 1 L plastic container, sealed, hardened for 1.5 h in a freezer (-80 ± 3 °C, Thermo Electron Corp., Revco, Cambridge, UK), and stored at -20 ± 2 °C (Skopec TMEF65XL-D freezer, Skopec Industries Ltd, NSW, Australia) for 24 h before analysis.

2.3. Overrun and melting properties

Three batches of each type of kiwifruit ice cream were produced for overrun and melting assessment (Arbuckle, 1986; Schmidt, 2004). The overrun was calculated using the equation of “% Overrun = (Vol. of ice cream – Vol. of mix used)/Vol. of mix used $\times$ 100%”. Melting behavior was assessed by withdrawing 120 g of ice cream from the freezer at -20 °C and putting it into a dish that was kept at 20 ± 2 °C (humidity $53 \pm 2\%$). The times at which the first drop of ice cream dripped and when complete melting had occurred were recorded. The volume of unmelted ice cream was recorded at 30-min intervals using a graduated cylinder. Melted ice cream was removed and weighed.

2.4. Total soluble solid content and pH values

The total soluble solid content was measured in triplicate using a handheld refractometer (Pocket Pal-1, Atago, Tokyo, Japan) at 20 °C, and expressed as °Brix. The pH was determined in triplicate using a pH meter (CG837, Schott Instruments, Germany) equipped with a glass electrode (850, Schott Instruments, Mainz, Germany).

2.5. Rheological analysis

The sample preparation procedure was as follows: ice creams were stored at -20°C for 24 h before ice cream tablets (diameter 25 mm, height 5 mm) were generated using a pre-cooled (-20°C) metallic and cutting cylinder. The ice cream tablets from each formulation were loaded onto the rheometer (oscillatory rheometry, Physica MCR301, Anton Parr GmbH, Graz, Austria). Profiled plate–plate geometry (diameter 25 mm) was used to avoid wall slip for rheological measurements, and plates were allowed to equilibrate with ice cream samples for 2 min at either temperature (-5 or -10°C) before testing to avoid problems associated with melting at the surface of the geometry. A Peltier hood attachment was used to prevent heat exchange with the environment. Amplitude sweep tests for determining the linear viscoelastic region were performed at a constant frequency of 10 s^{-1} and a strain range of 0.001 to 100%. The frequency sweep test was conducted between 0.1 and 1000 Hz at -5 and -10°C (gap setting, 2 mm). Data were acquired (30 measuring points for each analysis) and elaborated with Rheoplus V2.66 (Anton Paar GMBH, Graz, Austria), and the values with variations $<5\%$ were recorded. If the variation was $>5\%$, more duplicates were measured.

2.6. Preparation of polyphenol extracts using Accelerated Solvent Extraction (ASE)

Extraction of PPs from AFKW or ice cream sample was performed in triplicate using the Accelerated Solvent Extractor (ASE300, Dionex Corp., Sunnyvale, CA, USA). The testing sample (3 g) was mixed with diatomaceous earth (Celite®) (4 g) to facilitate homogenous mixing. The resultant mixture was packed into 34-mL extraction cells, and sequentially subjected to acetone, methanol and hexane extractions under N_2 (at 40°C and 1500 psi, with 5 min heating and 10 min static time). The obtained extracts were collected separately, concentrated using the Ultra-Low Cold Trap Centrivap® (Model 78100–01, Labconco Corp., Kansas City, MO), dried using a freeze drier (Telstar Cryodos –80, Telstar Industrial SL, Spain), stored at -80°C , and reconstituted for Folin–Ciocalteu assay and HPLC analysis.

2.7. Extraction of chlorophylls and carotenoids using Accelerated Solvent Extraction (ASE)

Extraction of chlorophyll and carotenoids of ice cream samples was performed in triplicate using the Accelerated Solvent Extractor (ASE300) unit (purging time, 90 s; flush volume, 60%; static time, 10 min for the 40°C extraction and 2 min for the 100°C extraction). Ice cream samples (4 g) were mixed with diatomaceous earth (Celite®) at a ratio of 1:1. Three extraction cycles were performed and all extractions were conducted using acetone in a minimized lighting environment. The extracts were collected and dried using an Ultra-Low Cold Trap Centrivap® prior to HPLC analysis.

2.8. Total extractable polyphenol content and total antioxidant capacity

A Solid Phase Extraction column was used to pre-treat the extracts derived from ASE, prior to the TEPC analysis, to eliminate the potential interference of ascorbic acid with the Folin–Ciocalteu assay. The first eluate from SPE was used for the analysis of vitamin C (Section 2.11). The eluates resulting from each flush with 95% methanol (in an aliquot of 5 mL) were collected for analysis.

TEPC values of the AFKWs and derived ice creams were analyzed in triplicate following the method of Singleton, Orthofer, and Lamuela-Raventos (1997). A calibration curve [Absorbance at 760 nm v. Catechin concentration (mg catechin equivalent (CtE)/mL)] was established for the extraction medium using a microplate reader (SpectraMax Plus 384, Molecular Devices, Sunnyvale, USA).

The ferric reducing antioxidant power (FRAP) assay (Benzie & Strain, 1999) was used to determine (in triplicate) the total antioxidant capacity. A calibration curve was established [Absorbance at 593 nm v. antioxidant capacity (mg Trolox equivalent/mL)].

2.9. High Pressure Liquid Chromatography (HPLC) profiling of individual polyphenols

Individual PPs of ASE extracts (Section 2.6) were analyzed in duplicate by HPLC following the method of Stevenson, Wibisono, Jensen, Stanley, and Cooney (2006). The HPLC analysis was run (at 280 and 530 nm) using a Shimadzu analytical HPLC with a column oven (C40-10ASVP), auto-sampler (SIL-10AF), vacuum solvent degas module and diode-array detector (SPD-M10AVP), fitted with a Synergi® Polar-RP ether-linked column ($250 \times 4.6\text{ mm}$, $4\text{ }\mu\text{m}$ particle size, $80\text{ }\text{\AA}$ ether-linked column, Phenomenex, Auckland, New Zealand). The mobile phases (A) acetonitrile + 0.1% formic acid and (B) acetonitrile/water/formic acid (5:92:3) were pumped at 1.5 mL/min at 45°C . The injection volume was $40\text{ }\mu\text{L}$. Individual PPs were identified based on their retention time and absorbance maximum (λ_{max}). External standards, catechin, epicatechin, quercetin, 2,4-dihydroxybenzoic, *p*-hydroxybenzoic, protocatechuic, salicylic, syringic, ferulic, *p*-, *m*- and *o*-coumaric acids were used to assist with PP identification.

2.10. High Pressure Liquid Chromatography profiles of chlorophylls and carotenoids

ASE extracts (Section 2.7) were analyzed in duplicate using the Shimadzu analytical HPLC set up as discussed (Section 2.9). The gradient system was established as described in Dwamena et al. (2009). Carotenoid profiles were monitored at 450 nm with a photo diode array 996 Waters detector set at 300–700 nm. Chromatographic peaks were identified by comparison with authentic standards of *beta*-carotene, lutein, and antheraxanthin, violaxanthin, and zeaxanthin (Sigma, Australia). Where other standards (including chlorophylls) were not available, detected compounds were tentatively identified according to their retention times and UV–vis spectra (Jeffrey, Mantoura, & Wright, 1997; Rodriguez-Amaya, 1999). The concentrations of such carotenoids were calculated using the lutein calibration curve.

2.11. Determination of L-ascorbic acid

Dried eluate (3 g) from each type of ice cream (see Section 2.8.) was dissolved in water (10 mL) to give a final concentration of 0.3 g/mL for analysis (in triplicate) (Association of Official Analytical Chemists, AOAC, 1990).

2.12. Statistical analysis

Replication in the experiment included three independent batches for each control or kiwifruit juice ice cream. At least three observations per analysis were done. Data were analyzed using one-way ANOVA (Minitab 15).

3. Results and discussion

3.1. Product characteristics of ice cream made with kiwifruit juice fraction

In this study, the AFKW from the green, gold or red kiwifruit had a pH of 3.5, 3.6 and 3.8, and a total soluble solid content of 14.9, 13.8 and 15.6°Brix , respectively. The ice creams derived from the green, gold and red kiwifruit had the natural color of each type of kiwifruit, appearing as light green, light yellow and pink, respectively. These

colors were the net result of the intrinsic opaque white color of milk and the natural kiwifruit pigments such as chlorophylls, carotenoids and/or anthocyanins (McGhie & Ainge, 2002; Montefiori, Comeskey, Wohlers, & McGhie, 2009; Montefiori, McGhie, Costa, & Ferguson, 2005; Montefiori, McGhie, Hallett, & Costa, 2009; Nishiyama et al., 2005).

The overrun of the ice cream containing green, gold and red kiwifruit AFKWs was 90.5, 94.8 and 96.8%, respectively. The overrun depends upon the fat, milk-solids-non-fat and solid content of the ice cream mix. The ice creams produced for this study had a fat content of 9.5%, which classifies them as a low-fat product (the US standard 21 CFR 135.110). An elevated fat content can enable a higher overrun, as more coalesced fat droplets are available to trap a greater amount of air bubbles in the ice cream (Abd El-Rahman, Madkor, Ibrahim, & Kilara, 1997). A change in the emulsification capability of milk proteins such as the amphiphilic property can alter their interactions with other components at the air-cell interfaces of ice cream, resulting in different overrun values (Barfod, Krog, Larsen, & Buchheim, 1991; Schmidt, 2004). The differences in the soluble non-starch polysaccharide, protein and/or sugar contents of the AFKW from the three types of kiwifruit would be responsible for the detected overrun values. For example, there were significant amounts of pectic polysaccharides in the AFKWs (Sun-Waterhouse et al., 2009b; Sun-Waterhouse, Wen, Wibisono, Melton, & Wadhwa, 2009a; Yuliarti et al., 2008). These pectic polysaccharides would have influenced the process of milk coagulation, rheological properties of the ice cream emulsion, and consequently the ice cream microstructure (El-Nagar et al., 2002; Fagan, O'Donnell, Cullen, & Brennan, 2006; Schmidt, 2004). An extremely low overrun indicates little air has been included, causing an excessively cold sensation in the mouth and lack of creaminess and smoothness. If overrun was too high, the ice cream would be frothy. Most countries (including New Zealand) set 100% as the legal overrun limit for ice cream.

The melting behavior of the three kiwifruit ice creams was similar (Fig. 1). It took longer for the ice cream containing a gold or red kiwifruit juice fraction (157 and 160 min, respectively) to melt completely than that with a green kiwifruit juice fraction (145 min). The green kiwifruit ice cream started to melt approximately 1–2 min earlier than the other two ice creams, and its melting rate was the greatest especially after 100 min. These results might be associated with fruit attributes – green is a different species to gold and red. The kiwifruit with green, gold and red flesh had different compositions and intrinsic enzymes (Dawes & Keene, 1999; Deters et al., 2005; Lesperance, 2009; Nieuwenhuizen et al., 2007).

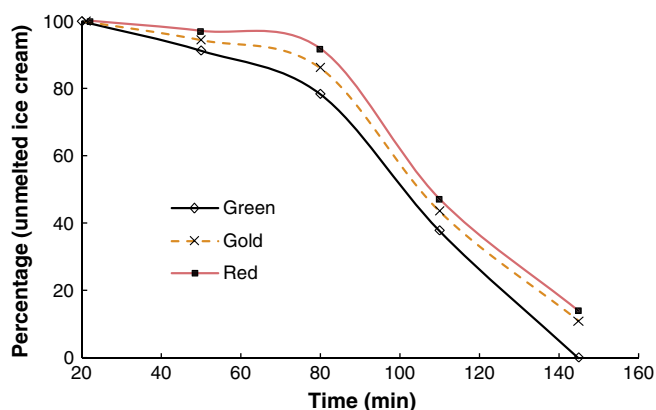


Fig. 1. Melting curves of three types of kiwifruit ice cream, (A) green kiwifruit ice cream, (B) gold kiwifruit ice cream and (C) red kiwifruit ice cream.

3.2. Rheological tests

Rheological properties indicate the flow behavior and product mouthfeel of ice cream. Amplitude sweep determined the linear viscoelasticity (around 0.01% strain, Fig. 2A). Generally speaking, the storage shear modulus G' of a material is time dependent, demonstrating the elastic behavior or the energy storage. In this study, there was a linear viscoelastic region under which G' was independent of time, amplitude of the oscillating strain or stress, and applied oscillating frequency: for green or gold kiwifruit ice creams in the strain range of 0.001 to 0.2, and for red kiwifruit ice cream in a wider strain range of 0.001 to 1. The change in G' of the three ice creams as strain was increased followed the same pattern but different amplitude. The G' ranked consistently, over the strain range of 0.001 to 100, in the decreasing order of green>gold>red. The lowest G' of the red kiwifruit ice cream might be associated with a stronger fat destabilization caused by the AFKW of red kiwifruit, compared with that of gold or green kiwifruit.

The relationship between G' and frequency over the range 0.1–1000 s^{-1} was not completely linear (Fig. 2B). The G' of all the ice creams exhibited a strong frequency dependence throughout the

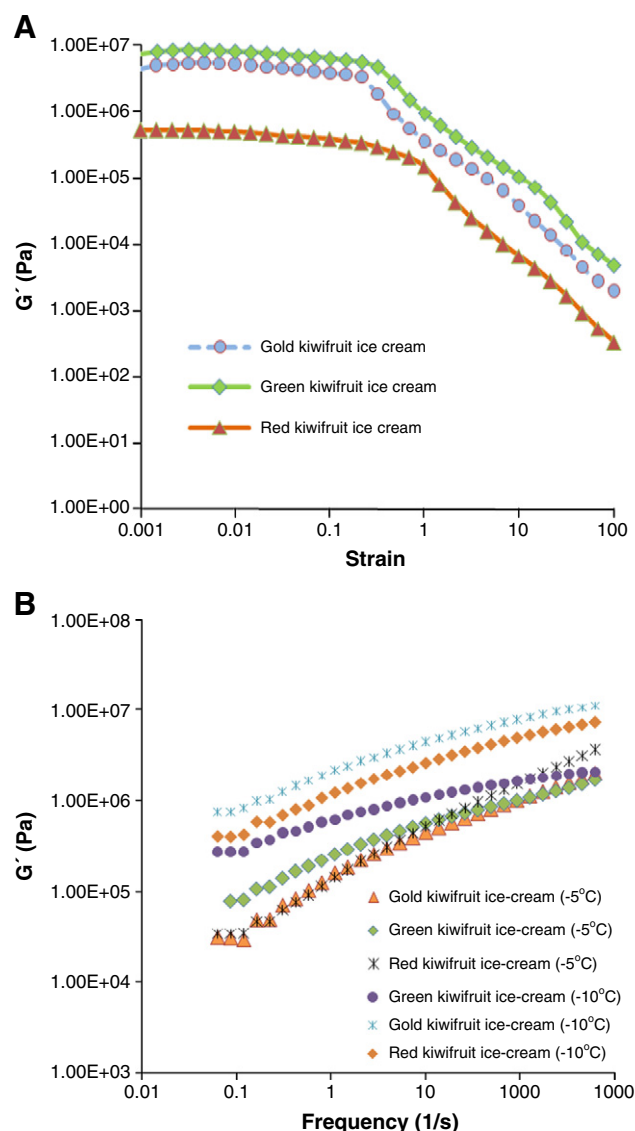


Fig. 2. Rheological analyses of three types of kiwifruit ice cream: (A), amplitude sweep plots; (B) storage modulus G' as a function of frequency.

range tested in this study. The type of kiwifruit juice had less effect at -5°C than at -10°C , and the average G' values of the ice creams at -10°C were significantly higher than those -5°C , indicating the importance of ice cream temperature in the magnitude of the G' values detected. Within the tested frequency and at the tested temperature of -10°C , G' values decreased in the order of gold-red > green, indicating a declining elastic component. At the tested temperature of -5°C , the G' values of gold and red kiwifruit formulations were nearly identical at a frequency $<10\text{ s}^{-1}$, however when the frequency was increased above 10 s^{-1} , the G' of red kiwifruit ice cream was highest. Although the contribution of each influencing factor to the G' is unknown, the fact that the overrun was similar for each formulation, and there was an apparent temperature dependence, implies that each type of ice cream had nearly the same ice phase fraction. Furthermore, the detected variation of 10 Pa in G' due to a difference of 5°C (between -10°C and -5°C) in ice cream temperature, possibly resulted from a change in the activation energies. As the temperature of the ice cream increased from -10°C to -5°C , the G' in this study decreased. The viscoelastic attributes of ice cream mixes are influenced by the size of fat globules, distribution of air cells, physical properties of protein-emulsifier film absorbed on the fat globule, as well as processing procedures such as homogenization and storage period (Velez-Ruiz & Barbosa, 1997). The degree of fat destabilization and the displacement of protein micelles from the fat globule surface affected the elasticity of ice cream (Adapa, Dingeldein, Schmidt, & Herald, 2000; Goff, Liboff, Jordan, & Kinsella, 1987; Hegenbart, 1996; Marshall & Arbuckle, 1996). Treatments having a higher degree of destabilization may have a higher elastic component (G'). The destabilized fat acts as a cementing agent and provides support to the air bubbles primarily lined by proteins, and air bubbles interact with fat in the surrounding medium to manipulate the elasticity component even in low-fat ice creams (Adapa et al., 2000; Goff et al., 1995).

3.3. Total extractable polyphenol content and antioxidant capacity of ice creams

Red kiwifruit ice cream contained the highest amount of TEPC (Fig. 3). The order of the TEPC in the three types of ice creams was the same as the AFKW ingredients (red > gold > green kiwifruit). The same volume of AFKW (500 mL) was added to each ice cream formulation, providing approximately 740, 845 and 925 mg PPs in the ice creams made with green, gold or red kiwifruit, respectively. Based on the obtained TEPC values (Fig. 3), the PP recovery for green, gold and red kiwifruit ice creams after the same ASE extraction process was calculated as 26.1, 27.5 and 43.1%, respectively. The similar PP recovery values of the green and gold kiwifruit formulations despite differing initial PPs in the formulation, suggest that extractability of

PPs from various kiwifruits differs in an ice cream matrix. This may have resulted from the PPs in the AFKW forming complexes with ice cream components such as milk proteins and polysaccharides during mixing and/or aging processing (Arts et al., 2002).

The trends for total antioxidant capacity (Fig. 4) were similar to those of TEPC (Fig. 2), indicating that the detected antioxidant capacity (after the removal of ascorbic acid by the SPE step) was mainly derived from TEPC (Rice-Evans, Miller, & Paganga, 1997). The SPE step was used during the analysis because kiwifruit has a high content of vitamin C but low concentration of PPs (Gil, Aguayo, & Kader, 2006; Wills & Greenfield, 1981). It is worth noting that in addition to the PPs and vitamin C in kiwifruits (Chun et al., 2005), polysaccharides may also contribute to the overall antioxidant capacity in a different way through interacting with antioxidants such as PPs and ascorbic acid (Sun-Waterhouse, Melton, O'Connor, Kilmartin, & Smith, 2008a; Sun-Waterhouse, Smith, O'Connor, & Melton, 2008b; Sun-Waterhouse, Smith, et al., 2008). Gold and green kiwifruits contain similar types of cell wall polysaccharides and a high proportion of cellulose, with the gold having a higher proportion of hemicellulosic polysaccharides and lower proportion of pectic polysaccharides (Sauvageau, Hinkley, Carnachan, & Sims, 2010).

3.4. HPLC phenolic and carotenoid profiles

Fig. 5 shows the HPLC chromatograms (at 280 and 530 nm wavelengths) for PPs extracted from the ice creams containing AFKW. The identified PPs in all the ice creams were those that are commonly found in green, gold and red kiwifruits, including protocatechuic acid, caffeic acid disaccharide/hexoside/derivative, dimethyl-caffeic acid hexoside, syringic acid, salicylic acid and/or o-coumaric acid (Dawes & Keene, 1999; McGhie & Ainge, 2002; Sun-Waterhouse, Chen, et al., 2009b; Sun-Waterhouse, Wen, et al., 2009a). PPs in green kiwifruit were previously reported as derivatives of coumaric and caffeic acids, chlorogenic acid, protocatechuic acid, epicatechin, catechin, procyanidins (B3, B2, or B4 and oligomers), the glycosides of quercetin (glucoside, rhamnoside, and rutinoside) and kaempferol (rhamnoside and rutinoside) (Dawes & Keene, 1999). In this study, ice creams made with aqueous fractions from green- and gold-fleshed kiwifruits had a higher concentration of caffeic acid disaccharide (peak 2) than those using red-fleshed kiwifruit. There were unresolved peaks at the retention times from 10 to 11 min in the 280 nm HPLC profile of the red-fleshed kiwifruit ice cream, which were possibly anthocyanins. The peak 9 at the retention time of 10 min in the HPLC chromatogram ($\lambda = 530\text{ nm}$) of the red-fleshed kiwifruit ice cream further confirmed the presence of anthocyanins (possibly cyanidin 3-galactoside or delphinidins). Anthocyanins such as delphinidin 3-[2-(xylosyl)galactoside], delphinidin 3-galactoside, cyanidin 3-[2-(xylosyl)galactoside], cyanidin 3-galactoside, and

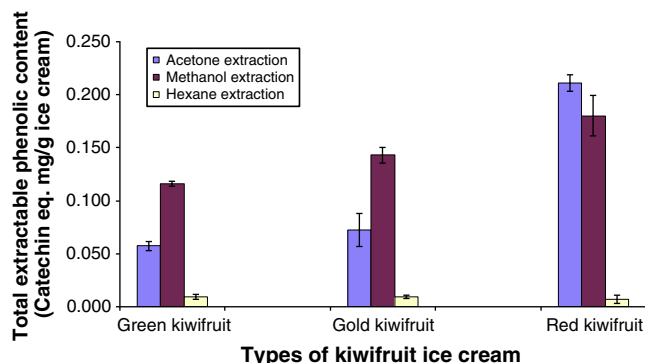


Fig. 3. Total extractable polyphenol content of ice cream made from kiwifruit with green, gold or red flesh. Error bars are the standard deviation of the mean.

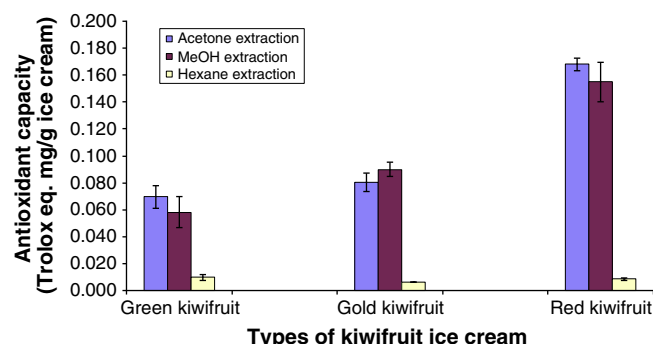


Fig. 4. Total antioxidant capacity of ice cream made from kiwifruit with green, gold or red flesh. Error bars are the standard deviation of the mean.

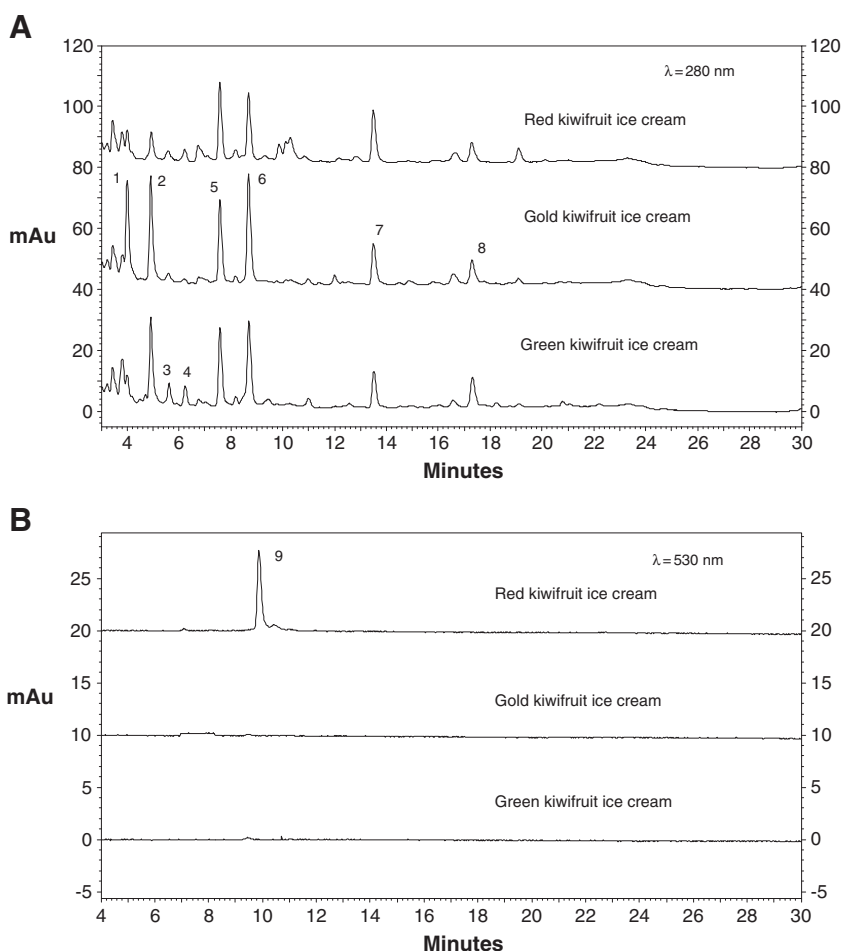


Fig. 5. Polyphenol profiles of ice cream (methanol fraction) made from kiwifruit with green, gold or red flesh, (A) $\lambda = 280$ nm, (B) $\lambda = 530$ nm: peak 1, unknown; peak 2, caffeic acid disaccharide; peak 3, caffeic acid derivative; peak 4, protocatechuic acid; peak 5, caffeic acid hexoside; peak 6 dimethyl-caffeic acid hexoside; peak 7, syringic acid; peak 8, salicylic acid and/or *o*-coumaric acid; peak 9, anthocyanins (possibly cyanidin 3-galactoside or delphinidins).

cyanidin 3-glucoside have been found in the same red-fleshed kiwifruit (Comeskey et al., 2009). These anthocyanins have been found to be beneficial to human health (Ghosh & Konishi, 2007; Kong, Chia, Goh, Chia, & Brouillard, 2003; Lila, 2004).

Fig. 6 shows the carotenoid profile ($\lambda = 450$ nm) of the ice cream made with AFKW from the green-, gold- or red-fleshed kiwifruit. Lutein and *beta*-carotene were identified as the major carotenoids in the three types of ice creams using the current analysis method,

although a big and broad peak around the retention time 8 min was detected in the profile of red-fleshed kiwifruit ice cream suggesting the presence of compounds with high polarity. The ice cream made with red-fleshed kiwifruit appeared to contain more species of greater hydrophilic carotenoid compounds (at higher concentrations) than the ice creams made with green- and gold-fleshed kiwifruit. The ice cream made with green-fleshed kiwifruit contained the least amount of lutein and a high concentration of chlorophyll (peak 3), while the

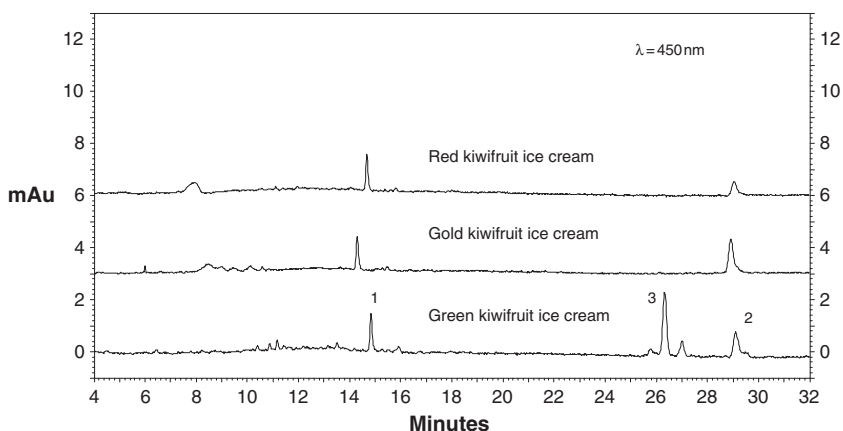


Fig. 6. Carotenoid profile of ice cream made from kiwifruit with green, gold or red flesh ($\lambda = 450$ nm): Peak 1, lutein; Peak 2, *beta*-carotene; Peak 3, a chlorophyll.

ice cream made with red-fleshed kiwifruit had the highest level of *beta*-carotene. Carotenoids are very susceptible to heat destruction and oxidation because of their highly unsaturated chemical structure (Stefanovich & Karel, 1982). The retention of both lutein and *beta*-carotene after ice cream making (including the pasteurization step) indicates these carotenoids may be stable in the ice cream matrix. The variations in the detected concentrations of lutein and *beta*-carotene may result from the initial concentration of the color pigments in the whole kiwifruit, timing of fruit harvest, storage conditions, and interactions of these compounds with the constituents in ice creams (Montefiori et al., 2005; Tavarini, Degl'Innocenti, Remorini, Massai, & Guidi, 2008; Yeum & Russell, 2002). When the kiwifruit juice fraction was mixed with milk to produce the ice cream mix, interactions among all the ice cream mix constituents could influence the structure and functions of active components initially present in the AFKW such as PPs and carotenoids. Interactions between these active components have been previously observed, for example, PP-induced complexation through intermolecular or intramolecular co-pigmentation effect (Rein & Heinonen, 2004).

3.5. Vitamin C content

Table 1 shows the initial vitamin C concentrations of the AFKWs from the green-, gold- and red-fleshed kiwifruits (0.92, 10.5, 0.76 mg/mL AFKW). In general, genotype or cultivar variations, physiological maturity, harvest factors, storage status, and analysis method utilized all contribute to the detected vitamin C content in fruits (Esti et al., 1998; Gil et al., 2006; Imeh & Khokhar, 2002; Kalt, 2005; Lee & Kader, 2000; Rassam & Laing, 2005; Tavarini et al., 2008). The ascorbic acid content of green and gold kiwifruits is reported as typically being between 536 and 600 mg, and 528–641 mg, per 100 g dry solids, respectively (Lesperance, 2009; Salunkhe, Bolin, & Reddy, 1991). The AFKW from red-fleshed kiwifruit in this study contained relatively lower concentrations of vitamin C possibly as a result of the longer storage period for the red-fleshed kiwifruit puree to accommodate fruit seasonal availability; green- and gold-fleshed kiwifruits, which ripen later in the season, were not stored for as long a period. Thus, the vitamin C content of the red AFKW could have been different from the detected values in this study, if the red-fleshed kiwifruit had been harvested later or stored for the same period as that for green- and gold-fleshed kiwifruits.

The detected vitamin C content in the ice cream made with green kiwifruit was significantly ($P < 0.05$) lower than those with gold- and red-fleshed kiwifruits (Fig. 7). Interestingly, for the same quantity of AFKW ingredients, the vitamin C content in green AFKW was significantly higher ($P < 0.05$) than that in the red AFKW (with a ratio of 1.21:1). This trend, however, reversed in the case of finished ice cream product. For the same amount of kiwifruit ice creams that contained the same quantity of added AFKW, the ratio of vitamin C content in the green and red kiwifruit ice creams was 1:1.46. Thus, the type of kiwifruit affected the stability of its vitamin C in the ice cream matrix. Furthermore, the result that the vitamin C derived from the kiwifruit was considerably retained in the final ice cream is encouraging, as an adequate quantity of ascorbic acid (also known as vitamin C) through dietary intake is very important for humans, who are not able to synthesize this compound but require it to

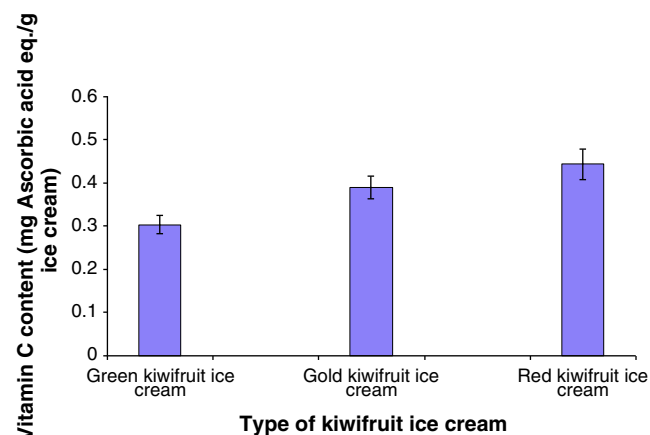


Fig. 7. Vitamin C content of ice cream made from kiwifruit with green, gold or red flesh. Error bars are the standard deviation of the mean.

neutralize reactive oxygen species (Davey et al., 2000; Ferraroni et al., 1994; Minnuni et al., 1992; Rababah et al., 2005).

3.6. Technical challenges during the ice cream preparation with kiwifruit juices

Preliminary trials were performed to identify the most appropriate volume of AFKW used in ice cream preparation; subsequently equal amounts of AFKW and milk (500 mL:500 mL) were selected. It was found that both pH pre-adjustment and pre-heating of the AFKW played critical roles in ice cream making. Use of AFKW introduced the potential for interactions among the kiwifruit juice components, milk proteins/fat and stabilizer during processing, and thus a change in the degree of fat agglomeration in ice cream (Schmidt, 2004). For ice cream making, agglomerated fat helps create ice cream structure: an increasing degree of fat agglomeration is associated with a reduced rate of melting and better ice cream shape retention (Schmidt, 2004). Any alteration in the physical form of proteins in ice cream, such as might be caused by AFKW addition, could cause a change in melting properties (Schmidt, 2004) and ice cream microstructure (Boode, 1992; Van Boekel, 1980; Van Dam, Watts, Campbell, & Lips, 1995). The AFKWs contained a significant amount of water soluble polysaccharides from kiwifruits with viscosity imparting properties (Sun-Waterhouse, Chen, et al., 2009b; Sun-Waterhouse, Wen, et al., 2009a; Yuliarti et al., 2008). It was reported (El-Nagar et al., 2002) that the addition of a soluble fiber polysaccharide such as inulin altered the viscosity of ice cream mixes and the texture of the resultant ice cream product. Soluble fiber polysaccharides play an important role in milk coagulation kinetics (i.e. the gel time and coagulum firming rates) (Fagan et al., 2006). Pectin has a pronounced effect on the microstructure of milk gels, and such an effect was determined by the concentration of pectin, e.g. pectin of 0.2–0.4% (w/w) significantly reduces gel times (Fagan et al., 2006).

Severe curdling of ice cream mix occurred and kiwifruit juice separated out during the pasteurization and aging steps when the AFKW was directly used for ice cream preparation (in the preliminary trials of this study). It was found that a two-step pretreatment of the AFKW (a pH pre-adjustment followed by heating at 55 °C for 1 min) prior to addition to the ice cream mix avoided curdling. Curdling of ice cream mix still occurred if pH was pre-adjusted but heating was not used. Heating at 40, 45 and 50 °C for 1–3 min was also insufficient to prevent curdling. Interestingly, the minimal pH adjustment for green kiwifruit, was lower than that for gold and red kiwifruits (4.7 compared to 4.9 respectively). The activity of enzymes like actinidin in the AFKWs (Bublin et al., 2004; Nieuwenhuizen et al., 2007; Nishiyama, 2007), change in the pH of ice cream mix caused by the

Table 1

Total extractable polyphenol and vitamin C contents in the aqueous fractions from green, gold and red kiwifruit purees.

Aqueous fraction (AFKW)	Green kiwifruit	Gold kiwifruit	Red kiwifruit
TEPC (CtE, mg/mL)	1.48 ± 0.05 ^a	1.69 ± 0.04 ^b	1.85 ± 0.02 ^c
Vitamin C (mg/mL)	0.92 ± 0.02 ^a	1.05 ± 0.02 ^b	0.76 ± 0.03 ^c

Data expressed as mean ± standard deviations of triplicate measurements. Different superscript letters in the horizontal rows indicate significant differences at $P < 0.05$.

AFKW acidity, processing procedure like pasteurization, and interactions between milk proteins and the PPs in AFKW, may have all contributed to this (Arbuckle, 1986; Fox, 1989; Holt, 1995; Gastaldi, Lagaude, & Tarodo De La Fuente, 1996; Gastaldi, Lagaude, Marchesseau, & Tarodo de la Fuente, 1997; Augustin & Udabage, 2007).

The green and gold kiwifruits differed in abundance and cysteine protease activity (Nieuwenhuizen et al., 2007). The total cysteine protease activity in the gold kiwifruit was found to be smaller than that of the green kiwifruit (Nieuwenhuizen et al., 2007). Gold 'Hort16A' and green 'Hayward' had similar levels of basic actinidin isoform, but the gold did not express an acidic isoform, KAct1a, which was predominant in the green (Nieuwenhuizen et al., 2007). Red kiwifruits are reported to have had a different profile of enzyme system compared with green and gold kiwifruits (Wang, Maddumage & Atkinson, 2009). Differences in the enzymes present in kiwifruit might mean variations in a number of enzymatic reactions e.g. actinidin-catalyzed hydrolysis of milk proteins and gelatin. In this study, gelatin, a class of proteinaceous substances, was used as the stabilizer, given its reported effect in improving elasticity, consistency and stability (Johns & Courts, 1977; Zhou & Regenstein, 2006). Enzymes including actinidin in the AFKW needed to be inactivated through heating prior to the AFKW addition to the ice cream mix (Katsaros, Katapodis, & Taoukis, 2009; Nam, Walsh, & Yang, 2006; Wilson & Burns, 1983).

The pH of natural milk is around 6.64, at which the milk casein micelles are very stable (Walstra, 1990). A decrease in pH results in an excess of positive ions (H^+) which can neutralize the negative charges around the casein micelles, thus decreasing the electrostatic boundary layers and resulting in casein micelles coalescing (Gastaldi et al., 1996). In this study, the pH of the AFKW of green, gold and red kiwifruit was 3.5, 3.6, and 3.8, respectively. When these AFKWs were added to the ice cream mix, the pH of milk decreased significantly to between 4.6 and 4.9 (the normal pH of ice cream is about 6.3) (Arbuckle, 1986), which reduced the steric and electrostatic repulsion between casein particles, and caused micellar aggregation (Gastaldi et al., 1996). A high acidity is also associated with decreased whipping rates and a less stable mix or possible coagulation during pasteurization and processing (Arbuckle, 1986) which is clearly undesirable.

Food process methods such as heating, acidification/alkali treatment, addition of stabilizer/emulsifier, and homogenization, are commonly used to optimize functionality of milk components (Augustin & Udabage, 2007). Heating can induce changes in milk, including inactivation of enzymes, denaturation of whey proteins ($>60^\circ C$), and alteration of casein micelles association (Donovan & Mulvihill, 1987; Fox, 1989; Holt, 1995). The extent of protein denaturation is determined by the temperature applied and pH during heating (Augustin & Udabage, 2007; Law & Leaver, 2000). A decrease in the pH of milk systems prior to heating leads to more association of the denatured whey proteins to the casein micelle (Corredig & Dalgleish, 1996; Oldfield, Singh, Taylor, & Pearce, 2000; Vasbinder & de Kruif, 2003). The pH adjustment before heating in this study might have altered the gelation properties of milk, causing differences in protein structures formed at the time of heating. Small changes in pH (even though the digits after the decimal point) were found to cause large difference in gel properties (Anema, Lee, Lowe, & Klostermeyer, 2004). Fine-stranded gels with good water-binding activity are formed when the milk protein is at a pH much above its PI and low ionic strength, while particulate gels with a loose network of large protein particles and low water-binding capacity, are formed when the pH is nearer the isoelectric point and at high ionic strength (Langton & Hermansson, 1992). Furthermore, the pH of the ice cream mix before pasteurization would have played an important role in the charge-related interactions between the polar PP compounds in the AFKW and the milk caseins/whey proteins (Haslam, 1998; Léonil et al., 1994; Perez-Jimenez & Saura-Calixto, 2006; Rawel et al., 2001;

Rohn et al., 2004; Wegrzyn et al., 2008). This explains the requirement of the minimal pH adjustment prior to pre-heating of AFKW for the ice cream making in this study.

4. Conclusions

Health beneficial phytochemicals such as dimethyl-caffeic acid hexoside, caffeic acid derivatives, protocatechuic acid, syringic acid, salicylic acid/*o*-coumaric acid, lutein and *beta*-carotene were commonly detected in the three kiwifruit ice creams, although with differences in the PP and carotenoid HPLC profiles. Only the ice cream made using red kiwifruit contained anthocyanins, and such ice cream had the highest level of TEPC and antioxidant capacity, reflecting the attributes of the AFKW ingredients. The vitamin C content in the green kiwifruit ice cream was the lowest, despite a higher vitamin C content in the AFKW ingredient, indicating that the type of kiwifruit affected the stability of vitamin C in the ice cream matrix. The G' decreased in the order of green>gold>red, suggesting that red kiwifruit AFKW might facilitate stronger fat destabilization and consequently lower elasticity in ice cream than gold or green kiwifruit AFKW. The ice cream containing green kiwifruit had a shorter melting time and lower overrun than the other two ice creams, which was associated with the detected difference in G' . The variations in the properties of ice creams reported here may result from the differences in the composition of three types of kiwifruit (e.g. the concentrations of PPs, vitamin C, pigments and the proteolytic enzyme actinidin), also the acidity of kiwifruit purees and the derived AFKWs.

It is possible to use a substantial amount of AFKW (e.g. at a level of 49% v/v) to produce ice cream: the final products have retained much of the natural colors as well as the unique vitamin C, PP and carotenoid antioxidant contents of various kiwifruit. Future work may include the exploration of alternative approaches to control the undesired enzyme activities in kiwifruit puree or its derived AFKW e.g. ultrafiltration and high pressure processing. Flavor was not a focus of this scoping study, although preliminary taste trials showed such kiwifruit ice creams had natural and pleasant flavor of the kiwifruit used. Formal consumer evaluation of this ice cream format would need to be conducted after the optimization of formulation and processing methods.

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