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Research Paper

Characterization of the ergometric properties of commercial bioactive dairy peptides

Luis E. Maya, Martin G. Scanlon *

Department of Food and Human Nutritional Sciences, University of Manitoba, Winnipeg, MB, R3T 2N2, Canada

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ABSTRACT

The thermodynamic properties of bioactive peptides provide insights into their functional behavior and their biological efficacy. We conducted precise analyses of the density, the ultrasonic velocity and the relative attenuation of serial dilutions of three commercial dairy peptides prepared by enzymatic methods. From these we determined the partial specific volume and the partial specific adiabatic compressibility coefficient for the peptides. At concentrations greater than ~2.5 mg mL^{-1}, the apparent values for specific volume and adiabatic compressibility were constant, differing between the three peptides at ±3% for specific volume and ±70% for compressibility. Both specific volume and adiabatic compressibility were highly dependent on concentration, indicating the importance of precise low concentration measurements to obtain correct values for these thermodynamic parameters. From these parameters it was apparent that restructuring of water molecules around the peptides (and their associated counterions) led to compact solutes that were also incompressible. These thermodynamic analyses are critical for understanding how the properties and the beneficial effects of bioactive peptides are influenced by their chemical environment.

1. Introduction

The two main protein components from milk, casein and whey, are reported to have several bioactive peptides within their structure that can be released during human digestion or during food processing (Cheung et al., 2015; Erdmann et al., 2008; Hartmann and Meisel, 2007; Korhonen and Pihlanto, 2006). Bioactive peptides exert several different beneficial health effects, including antihypertensive, antioxidative, antithrombotic, immunomodulatory, and hypocholesteloremic effects (Brandelli et al., 2015; Cheung et al., 2015; Erdmann et al., 2008; Saito, 2008).

Peptides, like many important biomolecules, have to be in an aqueous environment in order to perform their biological function (König and Boresch, 2009; Raschke, 2006). Aqueous solubility is primarily defined by the hydrophilicities of the amino acids at the surface of the peptide (Hedwig and Høiland, 2005; Raschke, 2006), taking into account any secondary and tertiary structures (Bellissent-Funel et al., 2016). The most favourable interactions with aqueous solvents are provided by charged and polar groups of the hydrophilic side chains (Raschke, 2006). Amino acids in the interior of structures formed by the peptide contribute very little to solvent affinity because of their limited solvent interaction (König and Boresch, 2009).

In order to understand the nature of peptide interactions with water molecules, especially those due to changes arising from conformational changes and interaction with ligands (Zhang et al., 2015), volumetric properties have been measured using ergometric principles (Chalikian and Breslauer, 1998; Chalikian and Filfil, 2003; Corredig et al., 2004; Durchschlag and Jaenicke, 1982; Gekko and Hasegawa, 1986; Jansens et al., 2016; Jansens et al., 2017; Murphy et al., 1998; Pfeiffer et al., 2008; Sarvazyan, 1991; Sarvazyan et al., 1979; Wang et al., 2006; Zamyatnin, 1984; Zhang and Scanlon, 2011). Properties that are work-dependent complement thermodynamic parameters such as specific heat capacity that are determined by calorimetric methods (Sarvazyan, 1991; Zhang et al., 2015).

Two principal ergometric (volumetric) parameters of a biomolecule are its partial specific volume and partial specific adiabatic compressibility coefficient. Changes in these parameters occur during conformational changes in the biomolecule, reflecting alterations in its intrinsic packing and the nature of its hydration (Chalikian and Filfil, 2003), so that ergometric assessments help us to understand conformational transitions, structural dynamics and the interactions of biomolecules as they accomplish their biological functions (Chalikian and Breslauer, 1998; Gekko and Yamagami, 1991; Jansens et al., 2016; Pfeiffer et al., 2008;

* Corresponding author.
E-mail address: scanlon@cc.umanitoba.ca (M.G. Scanlon).

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Taulier and Chalikian, 2001; Zhang et al., 2015). Interactions between water and protein molecules are especially important for protein dynamics, a primary determinant of the biological activity of proteins (Bellissent-Funel et al., 2016), but these interactions are also important to understand the behavior of peptides. For example, measuring the specific volume of peptides helps interrogate how peptides hydrate (Murphy et al., 1998).

Among several experimental options to measure the ergometric properties of biomolecular solutes, devices based on oscillatory principles are the primary means (Chalikian and Breslauer, 1998; Sarvazyan, 1991; Zhang et al., 2015). Density meters and ultrasonic resonators (based on oscillatory principles) have proven to be highly accurate, requiring only small amounts of a dilute solution and operating over a reasonable range of temperatures (Hedwig and Høiland, 2005; Zamyatnin, 1984; Zhang et al., 2015; Zhang and Scanlon, 2011). These methods can achieve a precision around 10^{-6} if temperature stability is maintained at 10^{-3} K (Kaatze et al., 2008; Sarvazyan, 1991).

From density measurements on the peptide solution, the derived apparent specific volumes permit the partial specific volume of the peptide to be determined from a limit extrapolation of a serial dilution (Gekko and Hasegawa, 1986; Zhang et al., 2015). A similar series of measurements of ultrasonic velocity permits (with the independent measurements of solution density) the adiabatic compressibility coefficient of the peptide solutions to be determined, from which the partial specific adiabatic compressibility coefficient for the peptide is ascertained from a limit extrapolation (Gekko and Hasegawa, 1986; Zhang et al., 2015). Both specific volume and adiabatic compressibility depend on the peptide's intrinsic properties and how its surface governs its hydration layers (Chalikian and Filfil, 2003; Murphy et al., 1998). For example, imperfect packing of the folded conformation of globular proteins makes their interior highly compressible (Gekko and Hasegawa, 1986). Conversely, the polarization of water molecules at the surface of peptides can substantially alter the partial specific volume and the partial specific adiabatic compressibility coefficient of the peptide (Chalikian and Breslauer, 1998; Chalikian et al., 1994; Gekko and Yamagami, 1991; Pfeiffer et al., 2008). A predominant factor is electrostriction, where the electric field of a dissolved solute polarizes the water molecules in the solvent shell surrounding the solute, so that they occupy less space and are less compressible. There are small or negligible effects on the peptide itself (Marcus, 2011).

To date, assessments of ergometric properties have been mostly applied to highly purified proteins or peptides, and have not been used yet to characterize the behavior of aqueous solutions of commercial food-derived peptides. Peptides generated by enzymatic scission of food proteins that meet a commercial application are processed in such a manner that they are typically >90% pure (Nielsen et al., 1997; Pouliot et al., 1999). Any non-peptide components in these commercial peptides can therefore alter our interpretation of biological function, particularly if they are ligands that dissociate from the peptide at low concentrations (Durchschlag et al., 1977; Vegarud et al., 2000). Accordingly, the objective of this study was to characterize the ergometric properties in aqueous solution of commercial bioactive dairy peptides at various concentrations in order to determine if these thermodynamic parameters provide insights into peptide behavior.

2. Materials and methods

2.1. Materials

Commercial bioactive dairy peptides were produced from whey proteins and referred to by the letters A, C, and D (Glanbia Nutritionals Inc., Fitchburg, WI, USA). The peptides were obtained by enzymatic hydrolysis and were dialyzed prior to spray drying into powders. The peptides are thus mixtures of peptides and any strongly associated ligands, especially counterions (Table 1). The only other material used in the experiments was ultrapure water, obtained from a Millipure system "Direct-Q 3". It was thoroughly degassed for 85 min using a vacuum pump at a vacuum between −25 and −30 mm Hg.

Table 1
Principal composition of the three commercial dairy peptides.

	Amount
"Protein", dry basis	>90%
Moisture	<5.0%
Fat	<0.7%
Minerals	<3.5%
Lactose	<1.0%

Additional chemicals and reagents were for cleaning the instruments: Ethanol 95% (Commercial Alcohol, Brampton, ON, Canada), Acetone 95% (Sigma-Aldrich, St. Louis, MO, USA), and Mucasol (Merz Hygiene GmbH, Frankfurt, Germany).

2.2. Preparation of serial dilutions of dairy peptides

Serial dilutions were prepared gravimetrically (for accuracy) using ultrapure and degassed water (UDWater) from two different peptide stock solution concentrations (20 mg g^{-1} and 15 mg g^{-1}), also prepared gravimetrically (both the mass of the peptides and the water). UDWater was added slowly over the walls of the beaker to avoid air incorporation. The stock solution was immediately covered to avoid water evaporation, which alters solution concentration, and was gently shaken for a specific period of time for each peptide using a rotary shaker (New Brunswick Scientific Company, Edison, NJ, USA) set at velocity 5. Time was selected based on peptide solubility. Solubility trials were done previously with each peptide, defining dissolution time as the time where no suspended particles were observed.

Each stock solution was serially diluted 5 times (1/32 dilution) to produce concentrations as low as 0.625 and 0.469 mg g^{-1}. In analyzing results, pairs of 20 mg g^{-1} and 15 mg g^{-1} stock solutions for each type of analysis (density or ultrasound) were used for limit extrapolations. Series for each analysis were prepared in triplicate, so that for each peptide, thermodynamic properties were effectively derived from 6 replicates (two stock solutions for each).

2.3. Density measurements

Density measurements were performed using a DMA 5000 density meter (Anton Paar GmbH, Graz, Austria). This equipment measures the dependence of the oscillation rate of a borosilicate glass U-tube containing the sample present within the tube, permitting highly accurate density measurements (Fortin et al., 2013; Zhang et al., 2015). Temperature is controlled to a precision of ±0.001 °C.

Measurements were performed from the lowest to highest concentration solution, starting with the fifth serial dilution (1/32). Three subsamples for each replicate serial dilution were measured, with an average density value reported. A new polypropylene/polyethylene syringe (3 mL) was used for each dilution. Density measurements were conducted at 24.985 °C.

2.4. Ultrasonic velocity and attenuation measurements

Ultrasonic velocity and attenuation measurements were performed for each concentration in the serial dilutions by propagating ultrasound through a liquid sub-sample in a ResoScan System (TF-Instruments GmbH, Heidelberg, Germany). The resonator unit works at a frequency range between 7 and 8.5 MHz, and is embedded in a metal block thermostat to provide temperature control with a precision of ±0.005 °C. To determine ultrasonic parameters, the resonance frequency of an ultrasonic pulse propagating in the peptide solution is measured by two parallel transducers located at different sides of a resonator cavity, where

one is the transmitter and the other the receiver (Kaatze et al., 2008; Sarvazyan, 1991; Zhang et al., 2015). The resonator unit has two sample cells, each with a maximum volume of 250 μL. For this study, one cell was used throughout as a reference (filled with fresh UDWater).

The equipment was cleaned at the beginning of all measurements with UDWater. Both cell cavities were rinsed thoroughly with UDWater several times. After the initial cleaning procedure, UDWater was added to both cell cavities and a "Resoscan Check" was performed to assure proper cleaning of the cells and that the ultrasonic velocity measurement was highly precise. The cleaning procedure was repeated until the velocity difference between cells was less than 0.025 m s^{-1}.

Measurements were performed from the lowest to highest concentration solution for each serial dilution. Three subsamples were analyzed for each concentration, with the velocity and attenuation averaged for that replicate. Ultrasonic velocity and attenuation measurements were carried out at 24.985 °C. Because of slight discrepancies in values for the absolute attenuation of water between the two cells, attenuation differences (peptide solution against UDWater) were recorded.

2.5. Amino acid analyses

Peptide composition determination was contracted out to an accredited laboratory. Samples of all three peptides were prepared for amino acid analysis by acid hydrolysis (AOAC method 982.30 (AOAC, 1990)). Performic acid oxidation prior to acid hydrolysis was conducted for Met and Cys analysis. An amino acid analyzer (Sykam, Eresing, Germany) was used for analysis (Kahindi et al., 2014).

3. Results and discussion

3.1. Density

The density (ρ) of the dairy peptide serial dilutions had very strong linear relationships against concentration (Fig. 1). Since serial dilutions were conducted gravimetrically, we report the actual concentration of each replicate, so that each "point" is actually three closely adjacent 'replicates'. The R^2 values for the linear regression of solution density against concentration were excellent, being 0.9999, 0.9997 and 0.9998 for A, C and D, respectively. In the lower concentration range (0.45–2.5 mg g^{-1}), linear relations between density and concentration were still very good, but R^2 values decreased to 0.9969, 0.9830 and 0.9846 for A, C and D, respectively. An increase in the variability of apparent specific volume determinations as solution concentration diminishes has also been reported for ionic solutions (Marcus, 2006).

3.2. Ultrasonic velocity and attenuation

As for density, the ultrasonic velocity (u) of serial dilutions of all three dairy peptides had strong linear relationships against concentration (Fig. 2). The relative differences in velocity between the three peptides were greater than those of density, especially between A and C. The R^2 values were outstanding, with values of 0.9999, 1.0000, and 0.9998 for A, C and D, respectively. At multiple dilutions there was less precision, with R^2 values dropping to 0.9980, 0.9977 and 0.9919 for peptides, A, C and D, respectively (0.45–2.5 mg g^{-1} range).

The relative ultrasonic attenuation of serial dilutions of dairy peptides A and C was more or less linear against concentration (Fig. 3), although anomalous effects upon dilution were apparent for peptide D at concentrations less than 2.5 mg g^{-1}. Variability in relative ultrasonic attenuation increased at low concentration, especially for dairy peptide D. Peptide C was distinguished by only a slight rise in its attenuation over the whole concentration range (approximately 10% of the concentration-dependent rate of increase in attenuation observed for the other two), with all peptides having low values compared to polymers.

Generally ultrasonic attenuation can be ascribed to attenuation caused by molecular or chemical relaxation processes, and attenuation caused by scattering (Corredig et al., 2004; Povey et al., 2011). Attenuation increases with molecular size (Corredig et al., 2004; Povey et al., 2011) and by processes that increase the bulk and shear viscosities of the solution (Dukhin and Goetz, 2009). The relative attenuation values suggest that the peptides in A and D are similar in size with structural features that dissipate acoustic energy more effectively than the smaller peptides in C. The increase in attenuation for solutions of D at low concentration is indicative of low concentration conformational changes in the peptides (Durchschlag et al., 1977; Durchschlag et al. ,1996). One putative mechanism is enhanced viscous dissipation associated with compressible structures in the peptides (Jansens et al., 2017), perhaps liberated upon release of attached counterions at low concentration.

3.3. Specific volume

The apparent specific volume, $\bar{v}_a$, of the dairy peptide serial dilutions was calculated from solution density (ρ) measurements as (Gekko and Hasegawa, 1986; Gekko et al., 2003; Zhang et al., 2015):

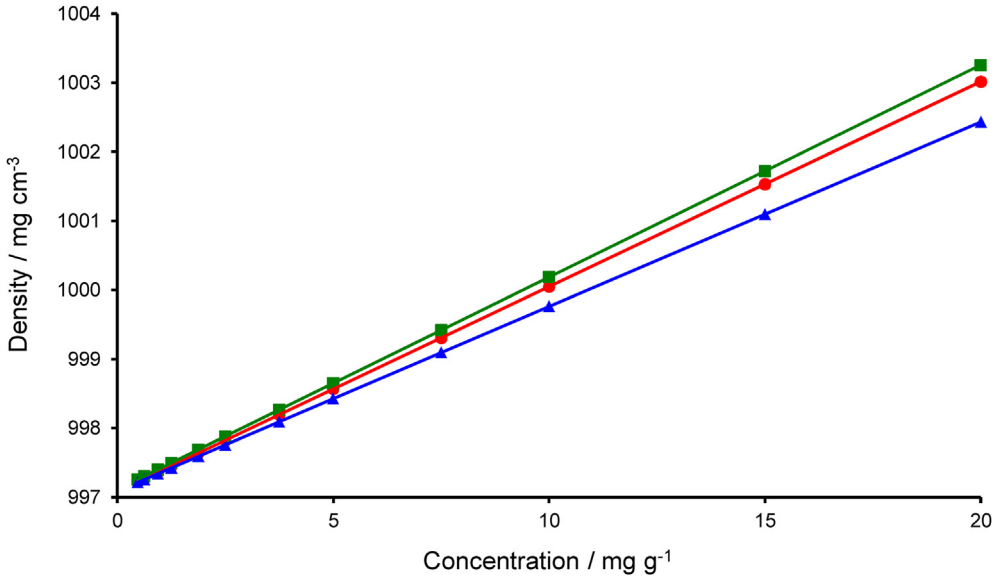

Fig. 1. Density of serial dilutions of dairy peptides, A (●), C (■) and D (▲).

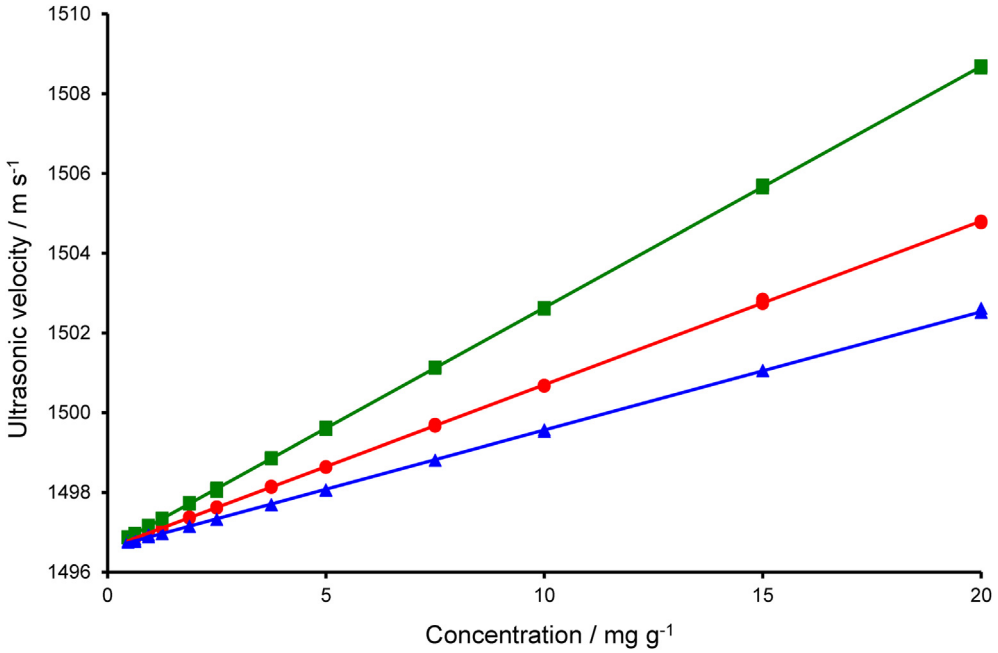

Fig. 2. Ultrasonic velocity of serial dilutions of dairy peptides, A (●), C (■) and D (▲).

$$\overline{v}_a = \frac{1 - [(\rho - c)/\rho_0]}{c}$$

where ρ_0 is the density of the solvent, and c is concentration of the peptides. The values as a function of concentration are shown in Fig. 4. Individual values for each "replicate" concentration is incorporated within the size of the symbols, demonstrating the highly precise nature of apparent specific volume determinations from the oscillating tube measurements.

The partial specific volume, $\overline{v}^0$, was calculated from extrapolation of the apparent specific volume values to zero concentration as $\overline{v}^0 = \lim_{c \to 0} \overline{v}_a$. This was performed using the non-linear curve fitting function of Origin 7.5 using all 36 data points to provide the best estimate of the intercept and its uncertainty. At concentrations above approximately 5 mg g^{-1}, the partial specific volume would be deduced to be identical to the apparent values. However, apparent specific volume values fall sharply as concentration diminishes. The differences between apparent specific volume values at higher concentrations and the partial specific volume were substantial for peptide C (0.095 cm^3 g^{-1}), but also significant for peptides D (0.050 cm^3 g^{-1}) and A (0.035 cm^3 g^{-1}). As a result, the peptides in D, that have an apparent specific volume some 0.03 cm^3 g^{-1} larger than A at higher concentrations, actually have a comparable partial specific volume (Table 2). This outcome is usually associated with changes in structure upon dilution that lead to severe electrostriction effects due to the polarizing influence of charged amino acids in the peptides (Chalikian and Breslauer, 1998; Chalikian et al., 1996), or the additive effect on the volume of released counterions that strongly polarize water molecules (Durchschlag, 1989).

Often, specific volume studies make use of higher concentrations than those of this study. For example, 20 mg mL^{-1} (Iqbal and Verrall, 1987) and 32 mg mL^{-1} (Gekko et al., 2009) have been used as lower limits in studies of the specific volume of various animal proteins, and 3 mg mL^{-1}

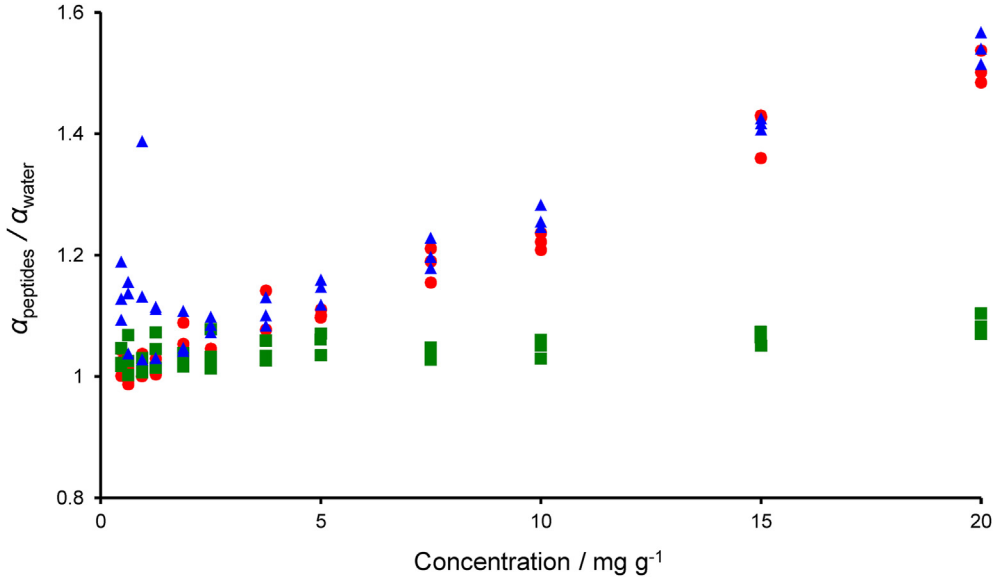

Fig. 3. Ultrasonic attenuation (α) of serial dilutions of dairy peptides, A (●), C (■) and D (▲), expressed relative to the ultrasonic attenuation of water.

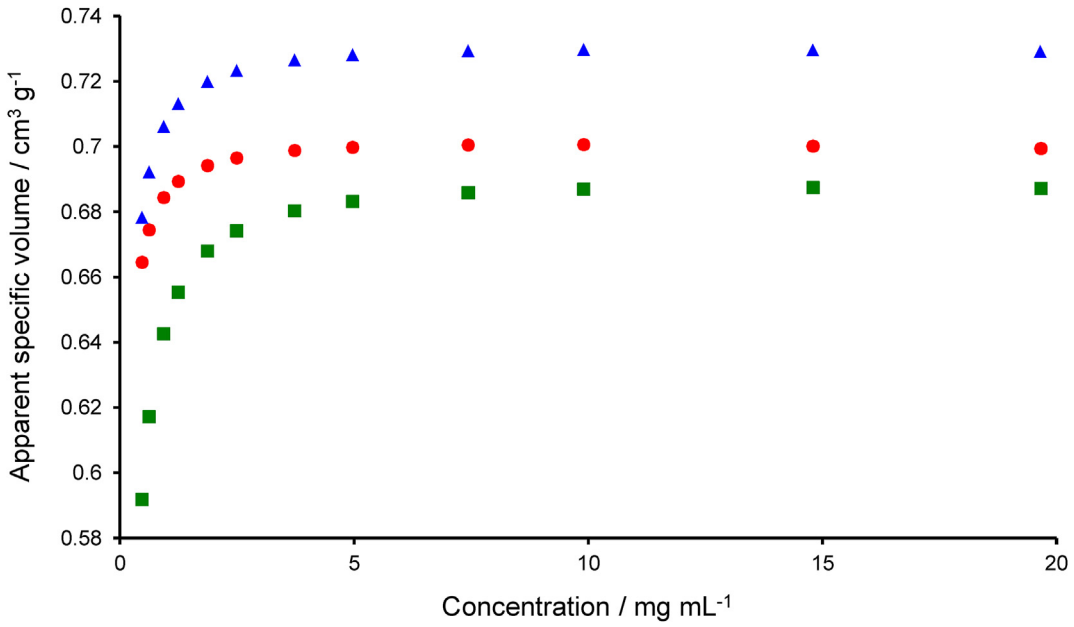

Fig. 4. Apparent specific volume of serial dilutions of dairy peptides, A (●), C (■) and D (▲).

Table 2
Partial specific volume of commercial dairy peptides.

	$\bar{v}^0$/cm^{3}g^{-1}
Dairy peptide A	0.63477 ± 0.000049[a]
Dairy peptide C	0.51540 ± 0.000096[a]
Dairy peptide D	0.63627 ± 0.000019[a]

[a] 95% confidence limits for n = 3.

was the limit in a study of the hydration of amino acids and peptides (Likhodi and Chalikian, 1999); 10 mg mL^{-1} was the lower limit for various sugar alcohol solutions in a determination of how partial specific volume affected sweetness perceptions (Lopez Chavez and Birch, 1997). This study clearly shows the need to conduct studies at concentrations below such thresholds in the analysis of commercial peptides due to the pronounced dependence of apparent specific volume as concentrations are lowered below approximately 2 mg mL^{-1}.

Partial specific volumes for most globular proteins at 25 °C range between 0.70 cm^3 g^{-1} and 0.75 cm^3 g^{-1} (Sirotkin et al., 2012; Durchschlag and Jaenicke, 1982; Gekko and Hasegawa, 1986; Murphy et al., 1998). For the amino acids glycine and alanine, apparent specific volumes at 25 °C were 0.578 cm^3 g^{-1} and 0.679 cm^3 g^{-1}, respectively, and no concentration dependence was observed (Chalikian et al., 1994). A slightly different value of 0.573 cm^3 g^{-1} was reported for glycine by Pfeiffer et al. (2008). In peptides, the type of amino acid strongly influences partial specific volume, especially for small peptides. For example, the partial specific volume of various glycine tripeptides (GXG) ranged from 0.497 cm^3 g^{-1} to 0.689 cm^3 g^{-1} (Likhodi and Chalikian, 1999; Schwitzer and Hedwig, 2005). Although the partial specific volumes of Table 2 are compatible with reported values for peptides, it is likely that the low value for peptide C, and the pronounced lowering of partial specific volume values as concentration is reduced, is due partially to the effect of counterions bound to the dairy peptides that are exposed or liberated at low concentration. The commercial dairy peptides used in this study had been dialyzed, but small amounts of salts and sugars persisted, "bound" to the peptides. Whey proteins, and peptides derived from these proteins, possess strong mineral binding properties (Thompson et al., 2009; Vegarud et al., 2000). These complexes can dissociate according to the pH and concentration of the solution (Dalgleish et al., 2005; Vegarud et al., 2000). Electrostriction effects by ions create very low values for partial specific volume: as low as 0.17 cm^3 g^{-1} for Na^+ (Imai, Nomura, Kinoshita and Hirata, 2002) and even negative values for highly polarizing ions such as Fe^{+++} (−0.78 cm^3 g^{-1} [Millero, 1971]). Therefore, conformational changes in the peptides that lead to exposure of the ions in A and D, and perhaps dissociation in C, induce strong polarizing effects on water molecules that lower the partial specific volume.

3.4. Compressibility

The adiabatic compressibility coefficient, β_S, of each solution was calculated from the Newton-Laplace equation:

$$\beta_S = \frac{1}{\rho u^2}$$

using the density (ρ) of the solution and its ultrasonic velocity (u) at a given concentration (Gekko and Hasegawa, 1986; Kaatze et al., 2008; Wang et al., 2006). The relative adiabatic compressibility coefficient, β_S/β_{S0}, which expresses the adiabatic compressibility of the solution relative to that of the solvent, β_{S0} (UDwater for this study), had strong linear relationships against concentration (results not shown). The value for β_{S0} is constant (44.7776 ± 0.00039 × 10^{-6} bar^{-1} at 24.985 °C, taken from the average of our water density and velocity measurements throughout the course of the study).

The apparent specific adiabatic compressibility, $\overline{K}_S$, which expresses the change in the apparent specific volume as a function of a change in pressure (Zhang et al., 2015), was calculated as:

$$\overline{K}_S = \beta_{S0}\left[\frac{\beta_S/\beta_{S0}\{(\rho - c)/\rho_0\}}{c}\right]$$

and this is plotted against concentration in Fig. 5. At higher concentrations, the peptides rank in terms of apparent compressibility as D more compressible than A, which is more compressible than C. As was observed for specific volume, the apparent specific adiabatic compressibility of the three dairy peptides was independent of concentration in the higher concentration range (5–20 mg mL^{-1}). However, at lower concentrations, the apparent specific adiabatic compressibility decreased rapidly, with the peptides' resistance to compression being greater as concentration diminished: the total decrease being about 0.3, 0.6, and

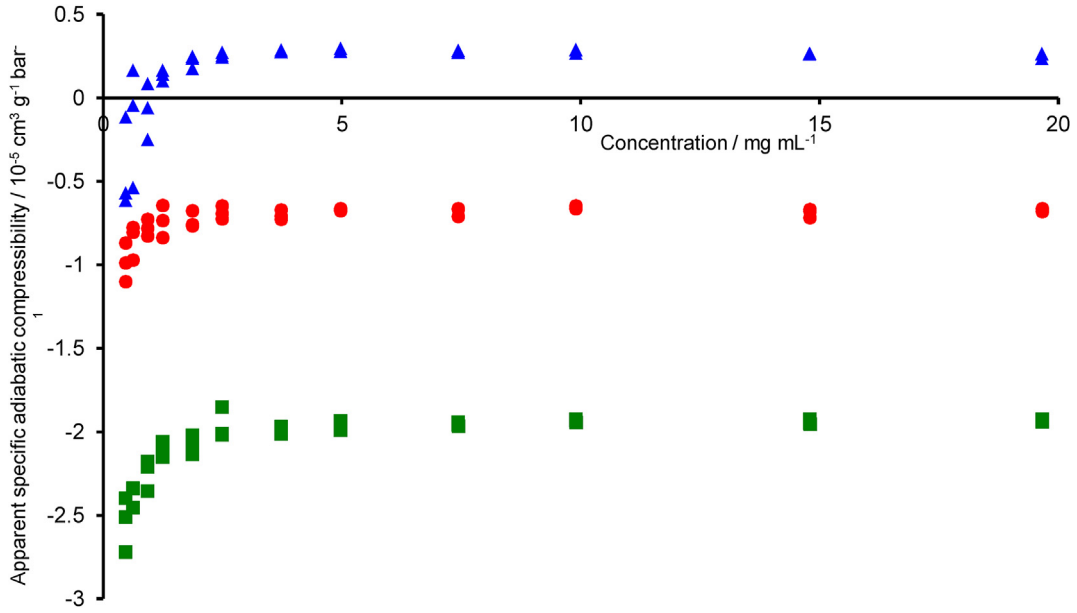

Fig. 5. Apparent specific adiabatic compressibility of serial dilutions of dairy peptides, A (●), C (■) and D (▲).

0.7×10^{-5} cm^3 g^{-1} bar^{-1} for A, C and D, respectively. Comparing Fig. 5 with Fig. 4, it can be deduced that the larger the peptides (including their associated hydration shell), the more compressible they are. In contrast to apparent specific volume measurements, $\overline{K}_S$ values were more variable at concentrations below 2.5 mg mL^{-1}, especially for the peptides in D.

The partial specific adiabatic compressibility coefficient, $\overline{\beta}_S^0$, which describes the relation between a molecule's apparent specific adiabatic compressibility at infinite dilution, $\overline{K}_S^0$, and its partial specific volume was calculated as:

$$\overline{\beta}_S^0 = \frac{\overline{K}_S^0}{\overline{v}^0} = \frac{\beta_{S0}}{\overline{v}^0} \lim_{c \to 0} \left[\frac{\beta_S/\beta_{S0} - \{(\rho - c)/\rho_0\}}{c} \right]$$

where the term $\frac{\beta_S/\beta_{S0} - \{(\rho-c)/\rho_0\}}{c}$ is extrapolated to zero concentration (Gekko and Hasegawa, 1986; Zhang et al., 2015), using the same procedure as for specific volume determination to ascertain the best estimate and uncertainty for $\overline{\beta}_S^0$. Values for $\overline{v}^0$ for each dairy peptide were taken from Table 2. The partial specific adiabatic compressibility coefficient values are shown in Table 3. All three dairy peptides have negative values, indicating that the peptides and their associated polarized water molecules are less compressible than the equivalent space that free water molecules occupy (Hedwig, 2006). It is possible that the dairy peptides at infinite dilution are even less compressible than indicated in Table 3 due to the sharp decrease in $\overline{K}_S$ values as concentration was lowered.

The partial specific adiabatic compressibility coefficient of several globular proteins are all positive (Chalikian et al., 1996; Gekko and Hasegawa, 1986; Iqbal and Verrall, 1987), ranging from $+1 \times 10^{-6}$ bar^{-1} to $+11 \times 10^{-6}$ bar^{-1}, as a result of the compressible nature of their interior (Gekko and Hasegawa, 1986). For amino acids, negative values for compressibility have been reported: -3.54×10^{-5} cm^3 g^{-1} bar^{-1} for glycine in water at concentrations of 3–4 mg mL^{-1} (Kharakoz, 1991) and -3.90×10^{-5} cm^3 g^{-1} bar^{-1} for the same amino acid in D_2O (Likhodi and Chalikian, 1999). Glycine is thus less compressible than the peptides in Fig. 5, although the $\overline{\beta}_S^0$ for the peptides in C is fairly close to the -61.2×10^{-6} bar^{-1} reported for glycine by Pfeiffer et al. (2008). Likhodi and Chalikian (1999) observed for oligoglycines that as peptide size increased, compressibility increased. For various glycyl oligopeptides, values between -1.22 and -2.19×10^{-5} cm^3 g^{-1} bar^{-1} were reported for $\overline{K}_S^0$ (Hedwig and Høiland, 2005), which for GDG would translate into a value of -31×10^{-6} bar^{-1} for its partial specific adiabatic compressibility coefficient, based on its reported partial specific volume (Schwitzer and Hedwig, 2005). It was noted (Hedwig and Høiland, 2005) that some glycyl oligopeptides had $\overline{K}_S^0$ values that were markedly dependent on concentration, becoming more incompressible as the solution was diluted and as side-chain carboxylic acid dissociation led to peptides that polarized water molecules around them. Similar effects are evident here for all three sets of dairy peptides. The low values for the partial specific adiabatic compressibility coefficient of dairy peptide C are surmised to be due to its low molecular weight (so no interior voids) and either its polar groups and/or associated counterions that are exposed or liberated as concentration is lowered (Kaatze, 2013).

Table 3
Partial specific adiabatic compressibility coefficient of commercial dairy peptides.

	$\overline{\beta}_S^0/10^{-6}$bar^{-1}
Dairy peptide A	-18 ± 5.9[a]
Dairy peptide C	-55 ± 13.6
Dairy peptide D	-14 ± 16.4

[a] 95% confidence limits for n = 3.

4. General discussion

From the ultrasonic and density measurements and the derivation of apparent and specific values of the thermodynamic properties of the three dairy peptides, two insights into the nature of these commercial peptides can be deduced. Firstly, considerable differences exist between measured values at concentrations greater than 5 mg mL^{-1} (~0.5%) and values at their limit dilutions. This means that caution must be exercised when making measurements of solutes such as specific volume, thermal expansibility and adiabatic compressibility at a particular concentration and using molality dependences to calculate thermodynamic values (Hedwig, 2006). Secondly, two dairy peptides had similar values for their measured properties, while the third differed considerably, comprised of compact molecules that were highly incompressible.

The amino acid composition of the three peptides is shown in Table 4. It can be seen that A and D are very similar in composition, contrasting

Table 4
Amino acid composition of the three dairy peptides.

Amino Acid	ASP	THR	SER	GLU	PRO	GLY	ALA	CYS	VAL	MET	ILE	LEU	TYR	PHE	HIS	LYS	NH3	ARG	TRP
Peptide A	10.8	6.4	5.2	17.6	6.8	1.4	4.7	3.1	5.1	1.6	5.5	9.5	2.6	2.8	2.2	9.4	1.5	1.8	2.1
Peptide C	5.2	8.5	4.9	7.9	2.2	1.1	7.0	0.2	8.8	6.6	6.6	20.1	3.1	4.9	2.4	6.1	1.3	1.8	1.1
Peptide D	10.5	6.4	5.2	17.2	6.8	1.4	5.0	2.6	5.6	1.5	5.8	10.2	2.6	2.9	2.1	9.2	1.5	2.0	1.6

markedly with the composition of peptides in C. In particular, C peptides are generally more hydrophobic, especially lacking in acidic side-chains found in A and D. The very slight increase in relative attenuation with increase in concentration for peptides C is also notable, indicative of a stable structure brought about by its hydrophobic nature. Amino acid composition does not always relate well to the partial specific adiabatic compressibility coefficient (Gekko and Hasekawa, 1986), so that it is the difference in structure between the peptides of A and D that account for differences in their compressibilities. Relative attenuation rises more substantially for the A and D peptides, so that relaxation phenomena in these peptides contribute to the increases in excess attenuation Pavlovskaya et al. (1992).

Peptide compressibility is governed by two contributions: intrinsic compressibility and the effect of the surface amino acids on the structuring of water around the peptide surface (Chalikian and Filfil, 2003; Murphy et al., 1998). The hydration contribution to compressibility is dominant for all the peptides, with the low values for compressibility caused by their enhanced electrostriction effects. As the dairy peptide solutions were diluted, an increase in the number of water molecules around exposed or dissociated salts, and restructuring of water around the peptide moieties where salts had been bound, caused greater interaction of the peptides with water molecules (Hedwig and Høiland, 2005; Kharakoz, 1991). In addition, possible conformational changes driven by the release of salts will lead to changes in thermodynamic parameters, as has been reported for lactoferrin (Chung and Raymond, 1993). Therefore, for the dairy peptides the partial specific adiabatic compressibility coefficient is mainly influenced by the properties of the water in the shell around the solutes. For smaller peptides, the overall contribution of the charged terminal amino acids on the electrostriction will more strongly influence compressibility (Hedwig and Høiland, 2005; Kharakoz, 1991; Likhodi and Chalikian, 1999), so that the attenuation result for peptide C supports the outcomes of Table 3. One indicator of a change in structure upon dilution that affects relative attenuation is its rise in Fig. 3 at low concentration for the peptides in D. A potential explanation for this excess attenuation is that the dissociation of ions leads to the peptides undergoing either internal motions not previously permitted or new dynamic interactions with proximal water molecules, both of which would lead to increased acoustic energy absorption.

In conclusion, ergometric analyses can be applied to commercial dairy peptides. Commercial dairy peptides are compact and incompressible, the more so as the number of hydrogen-bonding amino acids decreased. The less-refined nature of commercial food systems means that analysis concentrations have a bearing on interpretation of how thermodynamic parameters influence the functional properties of these biologically active molecules.

CRediT author statement

Luis Maya: Methodology, Data curation, Writing – original draft preparation. **Martin Scanlon**: Conceptualization, Supervision, Writing-Reviewing and Editing, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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