

Sugar replacement with zwitterionic plasticizers like amino acids

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ABSTRACT

In this paper, we investigate the potential of zwitterions like amino acids as alternative (bulking) sugar replacers. Commonly, polyols or oligosaccharides are taken as sugar replacers, but they are perceived as less attractive due to laxative problems. The choice of amino acids as alternative sugar replacers is inspired by their presence in Natural Deep Eutectic Solvents (NADES) and compatible solutes, next to carbohydrates. In these compounds, hydrogen bonding plays an essential role similar to sugar (replacers) in food. Because of the novelty of the topic, we first discuss at length NADES as a potential source of alternative plasticizers/sugar replacers. Subsequently, we perform a detailed analysis of their thermodynamic properties, to investigate whether amino acids and related zwitterions indeed have the appropriate properties for sugar replacement.

Our analysis shows that several zwitterions indeed have proper thermodynamic properties, which can make them good plasticizers and humectants. However, the use of several zwitterions can be restricted by their poor solubility, except for L-proline. Solubility problems can be circumvented by their use in mixtures of several sugar replacers, which enhances their solubility. The poor solubility makes it also difficult to characterize the glass transition temperatures of the pure solute $T_{g,g}$. Consequently, we have estimated them via extrapolation of the viscosity curves, which is shown to scale with T_g/T . We have taken T_g of the plasticizer solution as a measure of the hydrogen bond density, $n_{OH,eff}$.

That measure is shown to explain the melting behaviour of biopolymers like egg white and starch in glycine and L-proline solutions, similar to sugar (replacers). However, our measurements of the biopolymer melting show indications of phase separation at high moisture contents for both amino acid solutions as for carbohydrate solutions. Overall, the amino acids and other compatible zwitterions have very similar thermodynamic behaviour as sugars and their carbohydrate-based replacers. In proper combinations with alternative plasticizers, the zwitterionic compatible solutes can well be used in sugar replacement strategies for foods.

1. Introduction

In bakery products sugars provide a wide variety of functionalities, next to providing sweetness (Davis, 1995; Pareyt et al., 2009; Wilderjans, Luyts, Brijs, & Delcour, 2013; van der Sman & Renzetti, 2019). For many of these other functionalities, sugar is acting as a plasticizer. Hence, in sugar reformulation studies one often replaces sugar by polyols to provide the plasticizer functionality (Struck, Jaros, Brennan, & Rohm, 2014). However, polyols are not viewed by the food industry as the ideal solution to sugar replacement due to their laxative problems (Ghosh & Sudha, 2012; Lenhart & Chey, 2017). Hence, there is a demand for another class of food-grade, natural plasticizers that can provide similar functionality as sugar, but with fewer calories.

The functionality of sugar and its replacers in bakery and confectionery products can largely be characterized by their behaviour as (1) plasticizer and (2) humectant (Wilderjans et al., 2013; van der Sman & Renzetti, 2019). As the plasticizer, the sugar determines the viscosity and glass transition of the food product (van der Sman, 2013a, 2013b; van der Sman & Mauer, 2019). The strength of the plasticizing function of carbohydrates and polyols can be characterized by the number of hydroxyl groups available for intermolecular hydrogen bonding, $n_{OH,eff}$ (van der Sman, 2013b). Surprisingly, the volumetric density

of hydrogen bonds (which is also related to $n_{OH,eff}$) determines the melting behaviour of biopolymers, which encompasses the gelatinization of starch, denaturation/thermosetting of gluten, soy, sunflower protein and the melting of gelatin (van der Sman, 2016; van der Sman & Renzetti, 2019; van der Sman & Mauer, 2019).

We view that alternative plasticizers to be used as sugar replacers can be found in the class of ingredients used in the field of Natural Deep Eutectic Solvents (NADES) (Liu et al., 2018). This field takes inspiration from nature, and in particular from the so-called compatible solutes, which are accumulated in micro-organisms in high quantities in case of severe environmental stress like freezing or draught (Yancey, 2005; Choi et al., 2011). Often, sugars and polyols act as compatible solutes, which protect cell membranes and proteins in stress conditions, which dehydrate the cell. The protective properties of compatible solutes are largely based on their plasticizer properties, i.e. their capability to replace water via providing hydrogen bonding. They are called compatible solutes because they do not interfere much with the cells' metabolism (Chen & Murata, 2008). Next, to the neutral carbohydrates, micro-organisms are using also zwitterionic compounds as compatible solutes. They can be (1) amino acids like L-proline, glycine, L-alanine, and L-serine, or (2) amino acid derivatives like TMAO, betaine,

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choline, carnitine (having quaternary ammonium cation as a functional group), or (3) proline-like compounds like ectoine and hydroxyectoine (Yancey, Clark, Hand, Bowlus, & Somero, 1982; Yancey, 2005). For interested readers, we have written a short review on the use of NADES as alternative food plasticizers, which can be found as Supplementary Material to this paper.

In this manuscript below, we evaluate in particular the use of zwitterions like amino acids as alternative plasticizers, and in particular those having or modulating sweet taste. Amino acids having sweet taste are glycine, L-proline, L-alanine and L-serine (Schiffman, Sennewald, & Gagnon, 1981; Nishimura & Kato, 1988).

The use of NADES ingredients, like amino acids and their derivatives, as plasticizers is not novel. In food science, the sweet amino acids (glycine, L-lysine and L-proline) have been studied occasionally as plasticizer for biopolymers (Stein & Greene, 1997; Taylor, Taylor, Belton, & Minnaar, 2009; Selmin, Franceschini, Cupone, Minghetti, & Cilurzo, 2015; Ahmad, Samuelsen, Garvik, & Oterhals, 2018). It is observed that mixtures of amino acids with starch can show browning due to Maillard reactions. Weak organic acids are also studied as plasticizers for biopolymers (Cao, Yang, & Fu, 2009; Yoon, 2013; Niazi, Zijlstra, & Broekhuis, 2015). Betain has been proposed as a plasticizer for soy (Kweon, Slade, & Levine, 2017). NADES solvents have been considered as plasticizers for starch and cereal flour (Leroy et al., 2012; Abbott et al., 2014; Colomines, Decaen, Lourdin, & Leroy, 2016).

In pharmacy amino acid are also used as plasticizers for stabilization of mixtures with proteins (Mattern, Winter, Kohnert, & Lee, 1999; Al Husban, Perrie, & Mohammed, 2011; Izutsu, Yoshida, Shibata, & Goda, 2016). Traditionally pharmaceutical formulations contain a hydrophilic biopolymer like gelatin, dextran or maltodextrin with sugars or polyols. However, due to restrictions on sugar levels for obese or diabetes patients, amino acids are investigated as sugar replacers. Their application is challenged by the occurrence of phase separation and subsequent crystallization. In a way, it is quite comparable to phenomena observed in mixtures of starch and polyols (van der Sman, 2019a), which are plasticizers of comparable size as amino acids.

Small amino acids like glycine and L-alanine have a strong plasticizing effect, but they also crystallize easily, if compared to carbohydrates (Al Husban et al., 2011). Because of their solubility problems, amino acids are always used in a mixture of plasticizers (Mattern et al., 1999). It is shown, that amino acids in combination with sugars can produce a glassy system without crystals (Lueckel, Bodmer, Helk, & Leuenberger, 1998; Kasraian, Spitznagel, Juneau, & Yim, 1998; Forney-Stevens, Bogner, & Pikal, 2015). If the amount of amino acids exceeds the sugar, the amino acid will crystallize. A partial phase diagram is constructed for glycine/sucrose (Suzuki & Franks, 1993). Furthermore, in mixtures of plasticizers of different sizes, one can expect some synergy. One has observed that the stability of lyophilized sucrose-protein systems is enhanced by adding amino acids (Forney-Stevens et al., 2015), which is attributed to the hole-filling mechanisms, similar to glycerol in trehalose or gelatin matrices (Bellavia, Paccou, Guinet, & Hédoux, 2014; Ubbink, 2016).

The above short overview of the literature shows there is a potential for zwitterions like amino acids for use as novel sugar replacers. However, one must use them in mixtures of different plasticizers to avoid solubility problems. Furthermore, in these mixtures, one must be aware of undesired taste effects, Maillard reactions or crosslinking reactions. The effectiveness of the use of these zwitterions as sugar replacers, in their role of plasticizer and humectant, largely depends on their thermodynamic behaviour. For the remainder of this paper, we investigate this behaviour of the sweet amino acids, glycine, L-alanine, and L-proline, and also in combination with other plasticizers like sugars. For their behaviour as plasticizer and humectant, we are interested in (a) the water activity, (b) the solubility, (c) the glass transition temperature, and (d) their influence on the melting of biopolymers. The latter two properties are governed by the density of hydrogen bonds (van der Sman, 2013b, 2016; van der Sman & Renzetti, 2019).

To estimate the density of hydrogen bonds of the pure plasticizer, one must know about the glass transition of the dry ingredient — which is often difficult to measure. However, recently we have shown that the viscosity of semi-dilute solutions of the plasticizer can be used as an alternative means to determine that (van der Sman & Mauer, 2019). Hence, we will investigate also the viscosity of amino acid solutions and other solutions with zwitterions having a molecular structure similar to glycine. For our investigations, we have largely used data already available in the literature. Some novel data from our lab is used, which is explained in forthcoming papers (Renzetti, van den Hoek, & van der Sman, 2020).

The remainder of the paper is organized as follows. First, we describe the theories used in describing the thermodynamic behaviour of (sweet) amino acids. We have chosen for the Flory–Huggins theory for the thermodynamic description of amino-acids, because it is the framework we have used previously for the thermodynamic behaviour of carbohydrates (Van der Sman, 2017). Moreover, we intend to use this framework in the future to investigate the behaviour of complex mixtures of biopolymers, carbohydrates, amino-acids and water. In particular, we are interested in how amino acids as co-plasticizer influence the melting of biopolymers, like egg white or starch — for which the Flory–Huggins theory is a suitable theory (Van der Sman & Meinders, 2011). We are aware of the fact that in literature other theories from molecular thermodynamics are developed for describing amino acids, like UNIFAC (Gupta & Heidemann, 1990) and PC-SAFT (Held, Cameretti, & Sadowski, 2011). We conclude with a critical comparison of the Flory–Huggins theory with UNIFAC and PC-SAFT, indicating its limitations and advantages for describing food systems with predominantly hydrophilic ingredients.

2. Thermodynamic properties of sweet amino acids

Due to the poor solubility of amino acids in water, we have also analysed their properties in ternary mixtures with carbohydrates and water. Hence, the Flory–Huggins theories for ternary mixtures will be given. Note that, the binary case of a amino-acid solution is a special case, via setting the concentration of the carbohydrate to zero.

2.1. Flory–Huggins theory for ternary mixtures

Like ternary systems comprising two carbohydrates, we describe the thermodynamics of amino acid/carbohydrate/water systems using Flory–Huggins theory (Van der Sman, 2017). We will test the theory via the water activity of the amino acid solutions at room temperature. The glass transition of the pure amino acids is found to be lower than room temperature. Hence, the free-volume extension of the theory is not required in this study.

The chemical potentials of the two plasticizers $i = \{s, g\}$ and water $i = w$ are as follows (Van der Sman, 2017):

$$\begin{aligned} \frac{\mu_s}{N_s RT} &= \frac{\ln \phi_s}{N_s} - \left(1 - \frac{1}{N_s}\right) \phi_w + \left(\frac{1}{N_s} - \frac{1}{N_g}\right) \phi_g + \\ &\quad + (\chi_{sg} \phi_g + \chi_{sw} \phi_w)(1 - \phi_s) - \chi_{gw} \phi_g \phi_w \\ \frac{\mu_g}{N_g RT} &= \frac{\ln \phi_g}{N_g} - \left(1 - \frac{1}{N_g}\right) \phi_w + \left(\frac{1}{N_g} - \frac{1}{N_s}\right) \phi_s + \\ &\quad + (\chi_{sg} \phi_s + \chi_{gw} \phi_w)(1 - \phi_g) - \chi_{sw} \phi_s \phi_w \\ \frac{\mu_w}{RT} &= \ln(\phi_w) + \left(1 - \frac{1}{N_s}\right) \phi_s + \left(1 - \frac{1}{N_g}\right) \phi_g + \\ &\quad + (\chi_{sw} \phi_s + \chi_{gw} \phi_g)(1 - \phi_w) - \chi_{sg} \phi_s \phi_g \end{aligned} \quad (1)$$

ϕ_i is the volume fraction of compound i , N_i is the molar volume of compound i , relative to that of water, and χ_{ij} is the Flory–Huggins interaction parameter between compound i and j . Recall the relation between chemical potential of water and water activity:

$$\mu_w = RT \log(a_w) \quad (2)$$

The chemical potentials of the plasticizers μ_s and μ_g will be used to describe their solubility. In saturated solutions, the chemical potential of the dissolved plasticizers is equal to the chemical potential of the crystalline phase μ_X . This is also used to describe the solubility, which is the concentration/volume fraction where

$$\mu_g = \mu_X \quad (3)$$

The chemical potential of the crystalline phase of the solute is expressed as follows (Van der Sman, 2017):

$$\begin{aligned} \mu_X(T) = & -\Delta H_x \left(1 - \frac{T}{T_x}\right) - \Delta C_{p,x}^0 (T - T_x) - \frac{1}{2} \Delta \gamma_{p,x} (T^2 - T_x^2) \\ & - T (\Delta C_{p,x}^0 - T_x \Delta \gamma_{p,x}) \ln(T_x/T) \end{aligned} \quad (4)$$

T_x is the melting temperature of the pure amino acid, ΔH_x is the melting enthalpy at $T = T_x$, $\Delta C_{p,x}$ is the change in specific heat between crystalline and liquid phase of the pure amino acid at $T = T_x$, and $\Delta \gamma_{p,x}$ is the derivative of $\Delta C_{p,x}$ with temperature. Often, the melting temperature is difficult to measure independently, and the chemical potential is often approximated by the following expression: $\mu_X(T)/RT = A + B/T + C \ln(T)$.

We will also investigate the freezing behaviour of ternary systems. The freezing line will be computed using the condition for equilibrium: $\mu_w = \mu_{ice}$, the chemical potential of ice, which is given by:

$$\mu_{ice} = -\Delta H_m (1 - T/T_m) - \Delta C_{p,ice} (T - T_m) - T \Delta C_{p,ice} \log(T_m/T) \quad (5)$$

$\Delta C_{p,ice} = C_{p,ice} - C_{p,w} = -2100$ J/kg is the difference in specific heat of water and ice. $T_m = 273$ K is the freezing point of pure water. ΔH_m is the latent heat of fusion for ice. Given the ratio of the two plasticizers present in the unfrozen phase, we can compute the freezing temperature of the ternary mixture $T = T_f$ as a function of the volume fraction of water ϕ_w . Due to freeze-concentration ϕ_w will continuously decrease from its initial value while lowering the freezing point T_f , which is obtained via setting $T = T_f$ in the expression of μ_{ice} .

2.2. Glass transition

Frozen ternary mixtures of amino acids and carbohydrates are characterized by T_g' the glass transition of the maximally freeze-concentrated solution. Its value can be obtained from the intersection of the freezing line and glass transition line. Above, we have indicated how the freezing line can be computed using the condition $\mu_w = \mu_{ice}$. The glass transition line will be computed using the Couchman-Karasz relation:

$$T_g = \frac{y_g T_{g,g} \Delta c_{p,g} + y_s T_{g,s} \Delta c_{p,s} + y_w T_{g,w} \Delta c_{p,w}}{y_g \Delta c_{p,g} + y_s \Delta c_{p,s} + y_w \Delta c_{p,w}} \quad (6)$$

$T_{g,i}$ is the glass transition of the pure component i , y_i is the mass fraction, and $\Delta c_{p,i}$ is the change in specific heat at the glass transition of the pure component, measured in J/kg.K. The indices indicate the various compounds in the ternary mixture w for water, s for sugars, and g for polyols or amino acids. For water and sugars the parameter values are known. For many sugars and polysaccharides holds the apparently universal value of $\Delta c_{p,s} = 0.42$ kJ/kg K (Van der Sman & Meinders, 2011). However, for the smaller polyols holds a different universal value: $\Delta c_{p,g} = 0.85$ kJ/kg K (van der Sman, 2013b). Possibly, for amino acids there is also a universal value. Due to their small size, we expect this value to be similar to that of polyols.

2.3. Hydrogen bonding and viscosity

As shown recently, the melting behaviour of biopolymers is modulated by plasticizers, which can be quantified by $n_{OH,eff}$, which is a measure for the volumetric density of intermolecular hydrogen bonds (van der Sman, 2016; van der Sman & Renzetti, 2019; van der Sman & Mauer, 2019). $n_{OH,eff}$ is computed based on all plasticizers present, including water:

$$n_{OH,eff} = \phi_w \frac{N_{OH,w}}{v_w} + \phi_s \frac{N_{OH,s}}{v_s} + \phi_g \frac{N_{OH,g}}{v_g} \quad (7)$$

with v_i is the molar volume of compound i , and $N_{OH,i}$ is the effective number of hydroxyl groups per molecule available for hydrogen bonding. Originally, we have proposed to compute these numbers via the glass transition of the pure compound $T_{g,i}$ (van der Sman, 2013b):

$$\frac{1}{2} - \frac{1}{N_{OH,i}} = \frac{1}{2} \frac{T_{g,i} - T_{g,w}}{T_{g,\infty} - T_{g,w}} \quad (8)$$

$T_{g,w} = 139$ K is the glass transition temperature of water, and $T_{g,\infty} = 475$ K is the glass transition temperature of an infinitely long carbohydrate, i.e. polysaccharides including starch and dextrans (van der Sman, 2013b). Note, that for water follows $N_{OH,w} = 2$, which has effectively two hydroxyl groups.

If a plasticizer, like amino acids, has poor solubility in water, it is difficult to determine the glass transition of the pure compound. If the amino acid solution is cooled in the DSC, it will crystallize quite easily, because the mobility of water is still high if the solution gets supersaturated. Sugars have a high solubility, and the mobility of water in supersaturated conditions is sufficiently low, that the solution can remain supersaturated — as crystallization is kinetically hindered (Shalaev & Kanev, 1994).

Recently, we have shown for carbohydrates that viscosity can be an alternative means to determine $N_{OH,i}$ (van der Sman & Mauer, 2019). For (semi)-dilute solutions the viscosity at constant temperature is shown to be linear with $n_{OH,eff}$. We will investigate the viscosity of binary amino acid solutions, and ternary amino acid/carbohydrate solutions, and compare it with the master curve obtained for carbohydrates (van der Sman & Mauer, 2019).

Already very early, the viscosity of dilute aqueous solutions of plasticizers like sugars and amino acids has been compared to the Einstein correction for spheres suspended in the solution (Daniel & Cohn, 1936):

$$\eta = \eta_0 (1 + K\phi) \quad (9)$$

η_0 is the solvent solution and ϕ is the volume fraction of the suspended spheres. For hard spheres it holds that $K = 2.5$. The volume fraction can be computed from $\phi = v_i c$, with $v_i = M_{w,i}/\rho_i$ the molar volume, and c the molar concentration. It is claimed if the viscosity of plasticizer solutions is plotted against $K\phi$, also for higher concentrations, a master curve is obtained (Daniel & Cohn, 1936). It literature the above relation is often formulated in terms of the molarity of the solute, m , and it is extended as a quadratic relation:

$$\frac{\eta}{\eta_0} = 1 + Bm + Dm^2 \quad (10)$$

Already early the hypothesis is formed that K or the B-coefficient depends on the type and number of functional groups of the solute (Daniel & Cohn, 1936).

2.4. Hydrogen bonding and biopolymer melting

In previous papers we have shown that the melting behaviour of starch, gluten, egg white, gelatin and soy in presence of a variety of sugar (replacers) can be mapped to a single master curve (van der Sman, 2016; van der Sman & Renzetti, 2019; van der Sman & Mauer, 2019), if plotted against the effective volume fraction of plasticizers $\phi_{w,eff}$, which is defined as:

$$\phi_{w,eff} = v_w \frac{n_{OH,eff}}{N_{OH,w}} \quad (11)$$

For pure water, it holds that $\phi_{w,eff} = \phi_w$, and the master curve coincides with that of the binary biopolymer/water system. For the binary system the curve can be described by the Flory theory of biopolymer melting (Van der Sman & Meinders, 2011; van der Sman, 2016):

$$\frac{1}{T_m} - \frac{1}{T_{m,0}} = -\frac{v_U}{v_w} \frac{R}{\Delta H_m} (-\phi_w + \chi_{eff} \phi_w^2) \quad (12)$$

Table 1

Physical properties of amino acids characterizing moisture sorption, with χ_{gw} and $T_{g,s}$ values from this study.

Compound	$M_{w,g}$ (g/mol)	ρ_g (kg/m ³)	χ_{gw}	$T_{g,s}$ (K)
Glycine	75	1660	-0.2	220
L-alanine	89	1420	-0.2	225
L-proline	115	1370	0.0	250

Table 2

Data sets for a_w of amino acid solutions.

Compound	Temperature (°C)	Reference
Glycine	298	Smith and Smith (1937), Kuramochi, Noritomi, Hoshino, and Nagahama (1997), Ninni and Meirelles (2001), Tsurko, Neueder, and Kunz (2007), Marsh et al. (2017)
Glycine	288–303	Romero and González (2006)
DL-Alanine	298	Kuramochi et al. (1997), Marsh et al. (2017)
DL-Alanine	288–303	Romero and González (2006)
L-Proline	298	Smith and Smith (1940), Chirife, Fontan, and Scorza (1980), Ninni and Meirelles (2001)
L-Serine	298	Pinho (2007)

and

$$\chi_{eff} = \chi_0 + (\chi_1 - \chi_0)(1 - \phi_w)^2 \quad (13)$$

T_m is the melting temperature of the biopolymer in the mixture with water, which is the quantity predicted by the theory. $T_{m,0}$ is the melting point of the dry biopolymer, v_U is the molar volume of the repeat unit (monomer) of the biopolymer, ΔH_m is the molar enthalpy of melting (per mole of repeating units), χ_{eff} is the composition-dependent Flory–Huggins interaction parameter, with $\chi_0 = 0.5$ holding universally of all biopolymers (Van der Sman & Meinders, 2011; van der Sman, 2012). χ_1 is the interaction parameter in the limit of dry biopolymer systems, whose value does depend on the type of biopolymer. For example, starch has the value $\chi_1 = 0.8$ (Van der Sman & Meinders, 2011). For proteins, it is difficult to assign a value to v_U , as they are built from many types of amino acids. Hence, we assume it has the same value as for starch, as given in Van der Sman and Meinders (2011).

Furthermore, we note that food biopolymers are semi-crystalline. Hence, the above equation for the volume fraction ϕ_w refers only to the amorphous phase of the semi-crystalline polymer (Van der Sman & Meinders, 2011). We assume that the crystalline phase does not interact with water. Hence, a certain fraction of the biopolymer to be crystalline, and $\phi_p = 1 - \phi_w$ is thus the volume fraction of the amorphous polymer phase.

3. Results

3.1. Thermodynamic characterization

3.1.1. Water activity

In Fig. 1 we show the sorption isotherms for several amino acids. The used data sources are listed in Table 2. We have fitted the Flory–Huggins theory to their sorption isotherms, using Eq. (2) to relate the chemical potential of water μ_w to water activity a_w . We have made use of the material properties as listed in Table 1, which also shows the fitted interaction parameters χ_{gw} . The sorption isotherms in Fig. 1 are at different temperatures, which are listed in Table 2. Within the temperature range 288–303 K the moisture sorption is independent of temperature. It is likely that moisture sorption of amino acids is temperature independent over a wider temperature range, i.e. 273–373 K, similar to the case of carbohydrates (Van der Sman & Meinders, 2011; Van der Sman, 2017). Furthermore, we like to remark that the data from Ref. Marsh et al. (2017) extend beyond the solubility range of glycine and L-alanine. This is achieved via the use of small aerosol

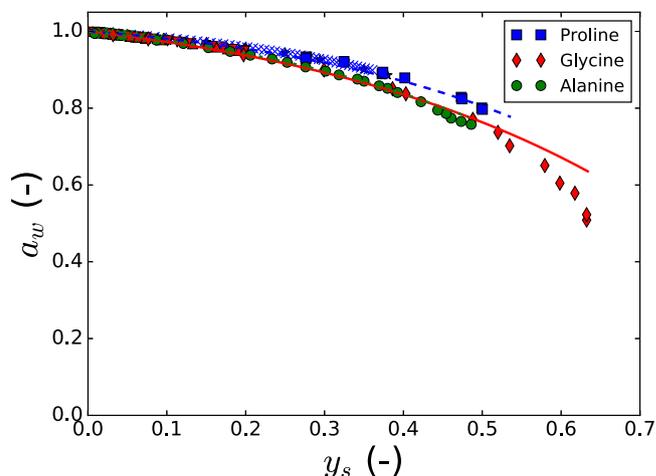


Fig. 1. Flory–Huggins theory fitted to sorption isotherm for proline, glycine and alanine. Fitted parameters are $\chi = 0.0$ for proline and $\chi = -0.2$ for glycine and alanine.

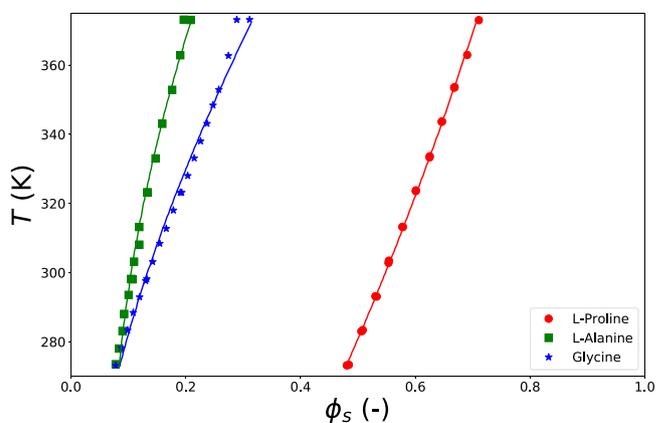


Fig. 2. Solubility lines of three amino acids in water. ϕ_s is the volume fraction of the solute, and T is the temperature.

droplets, with radii in the range of 4–30 μm , where crystallization is inhibited, but droplet curvature effects on water activity are negligible. This means a considerable extension of the data range for fitting the Flory–Huggins theory to the moisture sorption of glycine and L-alanine, which adds to the accuracy of the fitted interaction parameter χ .

The values of the interaction parameters are surprisingly compared to what we have found earlier for polyols and sugars (Van der Sman, 2017). A first explanation is the magnitude of the interaction parameter is quite dependent on the molecular size (van der Sman, 2019b). Furthermore, for small-sized molecules, amino acids have several functional groups, that can participate in hydrogen bonding: the amide group, and the carboxyl group. The latter can even be decomposed in a carbonyl and hydroxyl group, each capable of hydrogen bonding. The ratio of hydrogen bonding groups and the number of carbon atoms in the backbone is another important determinant of the Flory–Huggins interaction parameter (van der Sman, 2019b). For the investigated amino acids this ratio is particularly high for glycine and L-alanine, both showing even negative values — indicating high hydrophilicity.

3.1.2. Solubility

Solubility data are presented in Fig. 2. Data is obtained from Fasman et al. (1977), Amend and Helgeson (1997), Held, Neuhaus, and Sadowski (2010). The solid lines are obtained via predictions using Flory–Huggins theory, i.e. Eq. (3). Performing fitting with four parameters ($T_x, \Delta H_x, \Delta C_{p,x}, \Delta \gamma_x$) shows there is no unique set of parameters

Table 3
Parameters describing solubility of amino acids, with fitted $\Delta C_{p,x}$ and $\Delta \gamma_{p,x}$.

Compound	T_x (K)	ΔH_x (kJ/mol)	$\Delta C_{p,x}$ (J/mol/K)	$\Delta \gamma_{p,x}$ (J/mol/K ²)
L-alanine	608	22.0	92	-0.038
Glycine	569	21.0	35	-0.0090
L-proline	562	13.3	17	-0.0032

describing the solubility lines. Also, in literature one finds a wide variety of parameter sets, describing the solubility curve (Kuramochi, Noritomi, Hoshino, & Nagahama, 1996; Figueiredo, Da Silva, & Silva, 2013; Xu, Pinho, & Macedo, 2004). Consequently, we have performed fittings using fixed values for T_x , ΔH_x , using recent literature data from Held et al. (2011), Chua, Do, Schick, Zaitsau, and Held (2018). The fitted parameters are listed in Table 3.

3.1.3. Glass transition

Due to the poor solubility of amino acids, it is difficult to determine the glass transition amino acid solutions. Carbohydrates have much better solubility, and crystallization is often inhibited due to the high viscosity of saturated carbohydrate solutions. Consequently, the solutions remain supersaturated and solidify only if the glass transition is reached. For amino acid solutions, the crystallization is hardly inhibited due to the low concentrations of saturated solutions. They mostly form crystals rather than glasses.

Hence, we investigate whether the glass transition can be investigated for ternary mixtures, where the other solute is a carbohydrate and will provide a high viscosity of the solution, hindering the crystallization of the amino acid. In literature one finds data for the ternary mixture of glycine/sucrose/water (Chatterjee, Shalae, & Suryanarayanan, 2005). Sucrose is inhibiting the crystallization of glycine, beyond a critical ratio. The mixture is brought into the glassy state via freezing. In the experiment the maximum freeze concentration of ternary mixtures is determined, T'_g , which is the intersection of the freezing line and glass transition line, as shown in the exemplary phase diagram in Fig. 3.

Using the above Flory–Huggins theory we can compute the freezing line of the ternary mixture, via equating the chemical potential of water to that of ice: $\mu_w = \mu_{ice}$. Their expressions are given by Eqs. (1) and (4), using $\chi_{ws} = 0.49$ for sucrose, and $\chi_{wg} = -0.2$ for glycine. Furthermore, we assume that $\chi_{sg} = 0$. The glass transition line is computed using Couchman–Karasz, using values reported in literature, namely: $T_{g,g} = 280$ K for pure glycine and $\Delta C_{p,g} = 0.85$ kJ/kg K (Suzuki & Franks, 1993). The estimated error in the glass transition is $\Delta T_{g,g} = 5$ K.

T'_g is computed via solving for the intersection point of the freezing line and the glass transition line. The intersection is calculated for different ratios of glycine/sucrose. Experimental data on T'_g is available from various studies (Suzuki & Franks, 1993; Lueckel et al., 1998; Kasraian et al., 1998; Li & Nail, 2005), which is compared with our calculations, as shown in Fig. 4. In the figure, one can observe reasonable agreement between predictions and experimental data.

3.2. Viscosity

Unfortunately, there is no data available on glass transitions of other ternary amino acid/carbohydrate solutions. Hence, we will investigate whether $T_{g,g}$ of pure amino acids can be estimated from viscosity data, which is more widely available in the literature. In our previous study, we have shown that the viscosity of carbohydrate solutions is related to the ratio of T/T_g , which T the temperature of the solution (van der Sman & Mauer, 2019). In food science, it is common to use the WLF theory to describe viscosity near the glassy state. However, the WLF theory with universal constants cannot explain all viscosity data of carbohydrates (Recondo, Elizalde, & Buera, 2006; Sillick & Gregson, 2009; Dupas-Langlet, Meunier, Pouzot, & Ubbink, 2019). Yet, the scaling of

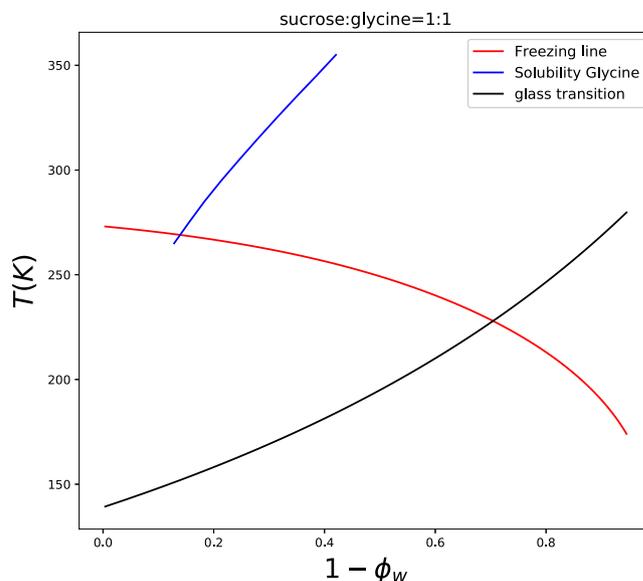


Fig. 3. Partial supplemented state diagram for 1:1 sucrose/glycine mixture, showing freezing line, glass transition, and solubility lines for glycine and sucrose. Sucrose inhibits glycine crystal formation. The intersections of the solubility lines and the freezing lines indicate the eutectic points for glycine and sucrose.

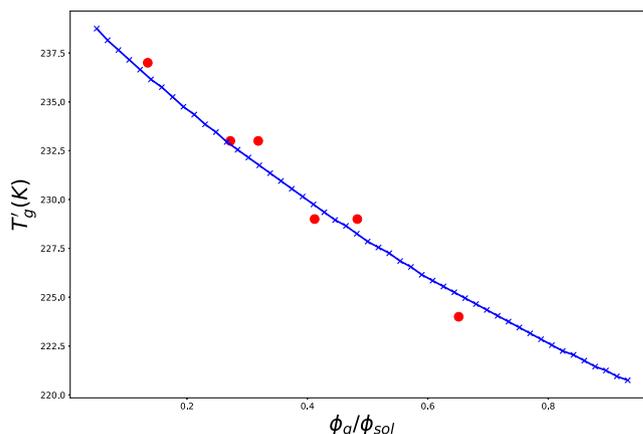


Fig. 4. T'_g of sucrose/glycine mixtures at different ratios. $\phi_{sol} = \phi_s + \phi_g$. Solid line is from the intersection of the predictions of the glass transition using Couchman–Karasz theory (with $T_{x,g} = 280 \pm 5$ K), and the freezing line using Flory–Huggins. Symbols represent experimental data from sources mentioned in the text.

viscosity with T/T_g can produce master curves for carbohydrates, over a wide range from pure liquid water to the glassy state $0.4 < T_g/T < 1.0$. Hence, we investigate whether the viscosity data of amino acid solutions also adheres to a master-curve if plotted against T/T_g .

For estimation of the glass transition of the solution, we will use the Couchman–Karasz relation, using $\Delta C_{p,g} = 0.85$ kJ/kg K $T_{g,g}$ of the dry amino acid will be estimated via collapsing the available viscosity data onto the master curve of polyols, as determined in our previous study (van der Sman & Mauer, 2019). As $\Delta C_{p,g} = 0.85$ kJ/kg K of amino acids is similar to that of polyols, we will collapse the amino acid data to the master curve of polyols, rendering an estimate of $T_{g,g}$ of the dry amino acid. The expression of the viscosity master curve is:

$$10 \log\left(\frac{\eta}{\eta_0}\right) = a_1 q + a_2 q^2 + a_3 q^3 \quad (14)$$

with $q = T/T_g - 0.35$, $\eta_0 = 0.231$ mPa.s, $a_1 = 3.9$, $a_2 = 7.9$, and $a_3 = 16.0$.

Data sources for the viscosity of amino acid solutions are listed in Table 4, which also includes some data from our lab. In Fig. 5 we show

Table 4
Data sources on viscosity of amino acids.

Compound	Temperature (°C)	Reference	Symbol	T_g
L-Alanine	5–35	Devine and Lowe (1971), Yan, Wang, Liu, and Lu (1999)	g*	259 ± 25
Glycine	5–35	Devine and Lowe (1971), Yan et al. (1999)	b.	220 ± 20
L-Proline	25	Belibağlı and Ayrancı (1990), Schobert and Tschesche (1978), Samuel, Kumar, Jayaraman, Yang, and Yu (1997)	cx	250 ± 3
Serine	5–35	Belibağlı and Ayrancı (1990), Yan et al. (1999)	y.	245 ± 20

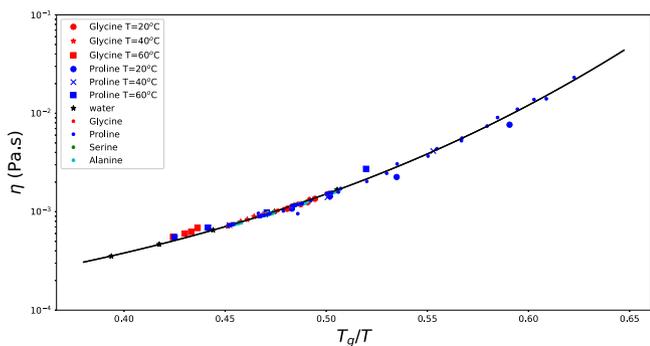


Fig. 5. Viscosity of amino acids as function of T_g/T , using our lab data (Renzetti et al., 2020) and literature data, as listed in Table 4.

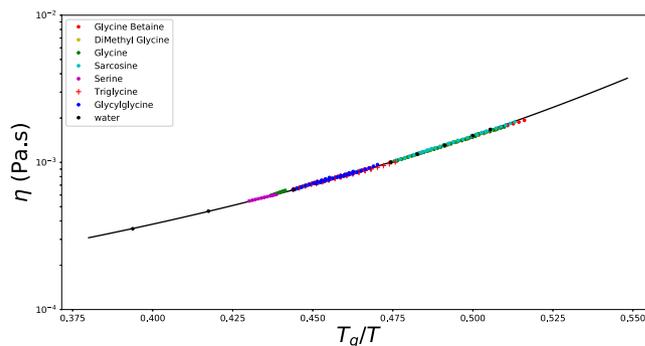


Fig. 6. Viscosity of zwitterions, similar to glycine, as function of T_g/T , using literature data as listed in Table 5.

how these experimental data are fitted to the master curve. The fitting is performed via minimizing the sum of least squares to $T_{g,s}$. The error in the estimation is computed using the covariance and Jacobian.

Compared to the literature data, the data from our lab show larger deviations from the master curve at low and high values of T_g/T . The literature data is often obtained via the Ubbelohde viscometer, while we have used a general-purpose rheometer from TA Instruments (Renzetti et al., 2020). Hence, we have used only the literature data to fit $T_{g,g}$ of the amino acids.

The resulting $T_{g,g}$ are also listed in Table 4. Note that the fitted $T_{g,g}$ of glycine (225 ± 20 K) is considerably lower than estimated from the freezing behaviour of glycine/sucrose solutions (280 ± 5 K), albeit that the error in the estimation of $T_{g,g}$ from the viscosity data is rather large. Below, when investigating the melting point depression of biopolymers, we will use both values for $T_{g,g}$ and observe which value provides the best prediction.

The viscosity of other zwitterions (as listed in Table 5), with structures similar to glycine, has been investigated. Only the B and D-coefficients from fitting Eq. (10) to the viscosity data have been reported. We compare these data with the above master curve for amino acids and estimate their $T_{g,g}$. The experimental data of these zwitterions compared to the master curve is shown in Fig. 6).

The investigated zwitterions are very similar, and they differ only in the presence or absence of some functional group. Hence, from the analysis of these viscosity data, we can learn how $T_{g,g}$ depends on the type and number of functional groups.

In Table 5 we have reported the fitted $T_{g,g}$ values and the estimation error. Despite large estimation errors in $T_{g,g}$ we do observe that additional methyl-groups hardly increase the $T_{g,g}$, while additional hydroxyl groups or peptide linkages will increase $T_{g,g}$. This can be explained by the fact that methyl groups do not contribute to hydrogen bonding, contrary to the hydroxyl groups and the peptide bond (van der Sman, 2019b).

3.3. Effects on biopolymer melting

Glycine and L-proline are investigated on their effect on protein denaturation and starch gelatinization. We compute $\phi_{w,eff}$ based on

the $T_{g,s}$ found in the previous sections. $T_{g,g}$ is converted to $N_{OH,g}$ using Eq. (8), and $n_{OH,eff}$ of the amino acid/plasticizer solutions is computed using Eq. (7). We note, that in these computations we take the $T_{g,g}$ values as obtained from the viscosity data, as listed in Table 5. This is also done for glycine, as the value $T_{g,g}=280$ K renders less agreement with other experimental data.

Mind that $n_{OH,eff}$ does not constitute the density of hydroxyl groups, but it must be regarded as the density of (intermolecular) hydrogen bonds, weighed against the strength of the hydrogen bond as imparted by the different functional groups (hydroxyl, carboxyl, amine, and peptide linkage). We investigate whether $n_{OH,eff}$ or rather $\phi_{w,eff}$ still explains the change in biopolymer melting. Below, we discuss the effect of different plasticizer solutions with amino acids on the denaturation of egg white proteins and gelatinization of wheat starch. Details on experimental methods are out of the scope of this paper, but they are discussed in detail in a forthcoming paper (Renzetti et al., 2020).

3.3.1. Egg white denaturation

In the same round of experiments, we have investigated egg white denaturation for the binary protein/water system for different moisture contents, and for ternary protein/plasticizer/water systems. The latter data set is extended with literature data. The first data set renders the desired master curve of the denaturation temperature, T_D , versus $\phi_{w,eff}$. The second data set allows us to compare the effect of amino acids and carbohydrates as modulators of biopolymers melting point. Results are shown in Fig. 7. We have listed the references of the used literature data sources in Table 6, together with the symbols used in Fig. 7 to indicate the various mixtures. For the labelling of symbols, we use the Matlab codes for plotting symbols.

The master curve for the denaturation of egg white is obtained from the Flory–Huggins theory of melting point depression using $T_m=400$ K, $\chi_1 = 0.8$, $\Delta H_m=52$ kJ/mol, and assuming that 60% of the protein is crystalline. These parameter values are estimated via fitting the theory to the experimental data for water.

In Fig. 7 we observe that the data for ternary mixtures largely follows the master curve for the binary protein/water system, confirming the hypothesis that egg white denaturation is also governed

Table 5
Data sources on viscosity of quarternary ammonium zwitterions, amino-acids and peptides.

Compound	Temperature (°C)	Reference	Symbol	$T_{g,g}$
Glycine Betaine	5–40	Tsangaris and Martin (1965), Tyrrell and Kennerley (1968)	r.	225 ± 15
Dimethyl Glycine	5–15	Tyrrell and Kennerley (1968)	y.	230 ± 10
Glycine	5–45	Tsangaris and Martin (1965), Tyrrell and Kennerley (1968)	g.	225 ± 20
Sarcosine	5–40	Tsangaris and Martin (1965), Tyrrell and Kennerley (1968)	c.	230 ± 15
Serine	30–50	Tsangaris and Martin (1965)	m.	245 ± 10
Glycylglycine	30–40	Tsangaris and Martin (1965)	b.	255 ± 15
Triglycine	30–40	Tsangaris and Martin (1965)	r+	260 ± 15
Alanine	15–25	Devine and Lowe (1971)	y+	225 ± 15
4-Aminobutyric acid	15–25	Devine and Lowe (1971)	g+	230 ± 20

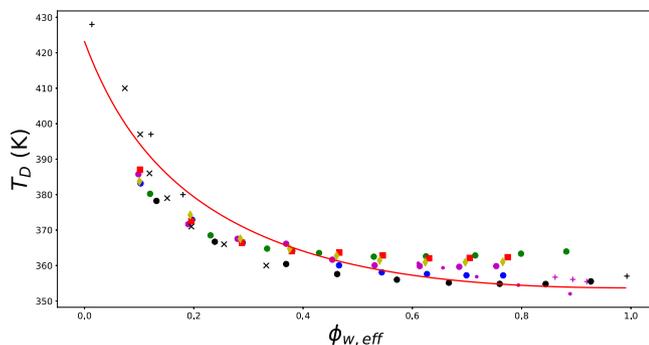


Fig. 7. Denaturation temperature T_D of ovalbumin in (spray) dried egg white as function of $\phi_{w,eff}$, the effective solvent volume fraction for various solutions with carbohydrates or amino acids. Used data sets and symbols are listed in Table 6. The solid line is obtained via fitting Flory–Huggins theory to the binary system of egg white/water.

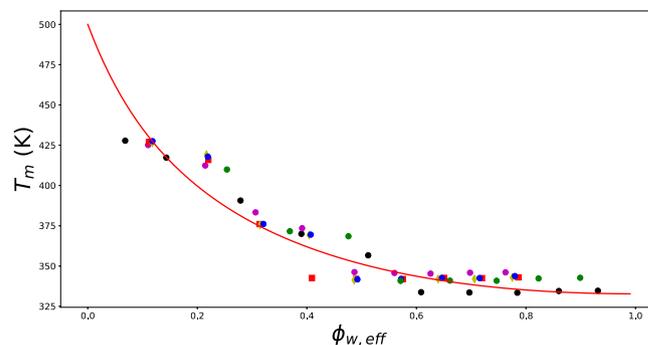


Fig. 8. Melting temperature of starch T_m as function of $\phi_{w,eff}$, the effective solvent volume fraction for various solutions with carbohydrates or amino acids. Similar symbols are as in Fig. 7. Solid line is obtained via fitting Flory–Huggins theory to the binary system of starch/water.

by hydrogen bond density as computed via $n_{OH,eff}$. At high moisture contents, we observe for both carbohydrates and amino acids deviations from the master curve, which we attribute to phase separation effects (Renzetti et al., 2020). This will be investigated in more detail in a future publication.

3.3.2. Starch gelatinization

Similarly, we have investigated the effect of glycine and L-proline of the melting temperature of starch, which is compared to data of some other carbohydrates and the binary starch/water system. The results are shown in Fig. 8. The solid line is the prediction by Flory theory of melting point depression, as obtained in our previous studies (Van der Sman & Meinders, 2011). The concentration of carbohydrates and L-proline in the solutions added to the starch are 30%, while glycine has a concentration of 15%, due to its limited solubility.

We observe that the experimental data collapse to a single curve, which follows the Flory–Huggins theory with $T_m=500$ K, $\chi_1 = 0.8$, $\Delta H_m=24$ kJ/mol, and assuming that 45% of the starch is crystalline.

Again at high values of $\phi_{w,eff}$ there are some indications of phase separation between the biopolymer (starch) and the solute (either amino acid or carbohydrate). From the above results for both egg white and starch, we conclude that (sweet) amino acids can be treated on the same footing as carbohydrates: they influence biopolymer melting via their effect on the hydrogen bond density.

4. Critical discussion on Flory–Huggins theory

As suggested by the reviewers we add a critical discussion of the Flory–Huggins theory in comparison to alternative molecular thermodynamic theories applied to amino-acids. These alternative theories fall within the field of chemical engineering called molecular thermodynamics, which has been pioneered by Prausnitz (Prausnitz & Tavares,

2004). We discuss two of these alternative theories, UNIFAC (Fredenslund, Jones, & Prausnitz, 1975) and PC-SAFT (Held, Sadowski, Carneiro, Rodríguez, & Macedo, 2013), which are exponents of two different classes of theories: (a) theories describing the excess free energy, and (b) theories describing equation of state, respectively. While UNIFAC describes only the activity coefficients of solutes and solvents, PC-SAFT can also describe other thermodynamic properties like density, boiling point, vapour pressures, and specific heat. These molecular thermodynamical theories are developed as an engineering tool to estimate the thermodynamic properties of chemical mixtures in the absence of experimental data.

UNIFAC theory describes interaction on the level of functional groups, thereby using the concept of local composition, as devised by Wilson (1964). This account for the non-random mixing of mixtures of compounds having different functional groups, leading to the clustering of similar groups at the molecular level as occurs in alcohol solutions. UNIFAC requires a lot of parameter values, which need to be fitted to a large amount of experimental data (Held et al., 2013). Parameters in UNIFAC are the Bondi areas and volumes of the various functional groups, entering the combinatorial part of the free energy, and two interaction parameters for each pair of functional groups. The main thesis of the UNIFAC theory is that these parameters are independent of the molecules containing these functional groups. If a reliable database of parameters is constructed the UNIFAC theory can be predictive for activity coefficients of a multitude of solutes and solvents, requiring no experimental input of mixture data. But, it is said to be limited if compounds have polar groups near to each other, such as polyhydroxy compounds as polyols and sugars (Prausnitz & Tavares, 2004).

PC-SAFT can account for chain-like molecules, which can have associative functional groups performing hydrogen bonding. The theory uses 6 pure-component parameters: (1) the (temperature-dependent) segment diameter, (2) the number of segments per molecule, (3) the

Table 6
Data sources used for denaturation of (dried) egg white and ovalbumin.

Reference	Solvent	Symbol
Rao and Labuza (2012)	Water	kx
van der Plancken, van Loey, and Hendrickx (2006)	Water	k+
Back, Oakenfull, and Smith (1979)	Sugar/polyol solutions	m.
Nafchi, Tabatabaei, Pashania, Rajabi, and Karim (2013)	Monosaccharide solutions	m+
Renzetti et al. (2020)	Water	ko
	L-proline solution	bo
	Glycine solution	go
	Sucrose solution	mo
	Glucose solution	rs
	Xylitol solution	yd

dispersion energy (for vanderWaals interaction), (4) the number of association sites, (5) the association energy, and (6) the association volume. For water these parameters are well known, assuming 4 association sites. PC-SAFT has common mixtures rules for computing parameters for mixtures for pure-component data, but also requiring the binary interaction parameter k_{ij} - which can be temperature-dependent.

UNIFAC is applied to variety of amino-acids and small peptides (Kuramochi et al., 1996; Ninni & Meirelles, 2001; Figueiredo et al., 2013), and carbohydrates (Ninni, Camargo, & Meirelles, 2000; Ferreira, Brig-nole, & Macedo, 2003). UNIFAC interaction parameters between polar groups often have to be readjusted if solutes contain multiple strongly polar groups, like OH or COOH groups. These hydrogen bonding groups show strong cooperative effects (Maffia & Meirelles, 2001).

PC-SAFT has been applied to amino-acids, and dipeptides (Cameretti & Sadowski, 2008; Grosse Daldrup, Held, Ruether, Schembecker, & Sadowski, 2010; Held et al., 2011). Amino-acids are assumed to have 2 association sites for each hydroxyl, amino- and carboxylic group. A large variety of amino-acids different amino-acids and dipeptides have been characterized (Lee & Kim, 2010; Held et al., 2011). It is shown that the number of segments is linear with the molar weight, and association parameters can be assumed constant for all amino-acids. PC-SAFT is also applied to small carbohydrate solutions (Held et al., 2013). The number of association sites is assumed equal to twice the number of OH-groups, all having the same association energy and volume. k_{ij} between carbohydrate and water, is linear dependent on temperature. k_{ij} between carbohydrates is assumed zero.

UNIFAC and PC-SAFT is often used for describing solid-liquid equilibrium for amino-acids or carbohydrates (Fuchs, Fischer, Tumakaka, & Sadowski, 2006; Grosse Daldrup et al., 2010; Held et al., 2013). The parameters required for the chemical potential of the crystalline/solid phase does not follow from the thermodynamics theories describing the activities of solutes and solvent in the liquid phase. The above equation, for the chemical potential of the crystalline phase, Eq. (4), holds for all three considered theories. The parameters within this equation must be determined via fitting to experimental data. However, it is said that solubility is largely determined by the temperature dependency of the parameters characterizing the crystalline phase, as solute activity coefficients are near unity. If one assumes temperature-dependent specific heat, ΔC_p , there are often multiple parameter sets, that can describe solubility data, as observed throughout literature.

Combined with dissociation equilibrium and Debye-Huckel the solubility of amino-acids at different pH and temperature of amino-acids are predicted (Fuchs et al., 2006; Lee & Kim, 2010). In UNIFAC the charged groups are defined as separate functional groups, with newly estimated interaction parameters (Figueiredo et al., 2013).

Using parameters established from binary solutions, predictions can be made for the solubility of ternary mixtures involving two amino-acids and water, or one amino-acid and two solvents (Fuchs et al., 2006; Held et al., 2013). For PC-SAFT the interaction parameter between two amino-acids, k_{ij} , is often found to be non-zero and temperature-dependent (Grosse Daldrup et al., 2010). Extending the theory with Debye-Huckel ternary mixtures involving salts can be described (Held,

Reschke, Müller, Kunz, & Sadowski, 2014). UNIFAC is successful in predicting solubility of carbohydrates in solvent mixtures, like water and alcohol. Water activity is computed of ternary mixtures, involving two carbohydrates (Ninni et al., 2000).

Flory-Huggins (FH) theory is also regarded by Prausnitz as a molecular thermodynamic theory, which he applied to swelling of polymeric gels (Hino & Prausnitz, 1998). FH theory is more simple than other molecular thermodynamic theories like UNIFAC and PC-SAFT. Flory-Huggins theory only assumes random mixing. Furthermore, the description of activity coefficients of solutes and solvents requires only the estimation of a single parameter, χ .

Nevertheless, FH theory is successfully applied to hydrogen bonding materials like biopolymer solutions (Hancock & Zograf, 1993; Shamblin, Hancock, & Zograf, 1998; Zhang & Zograf, 2001; Ub-bink, Giardiello, & Limbach, 2007; Van der Sman & Meinders, 2011) and gels (Van der Sman, 2015; Li, Zhao, Chen, & Mercadé-Prieto, 2016), solutions of carbohydrates (He, Fowler, & Toner, 2006; Van der Sman, 2017), and aqueous mixtures of both biopolymers and carbohydrates (Zhang & Zograf, 2001; van der Sman, 2013a), and even more complex mixtures involving salts (via extension with Debye-Huckel or even Pitzer theory) (van der Sman, 2012; van der Sman, Houlder, Cornet, & Janssen, 2020). FH theory is also applied to describe solubility of carbohydrates in water, and also in ternary systems of two carbohydrates in water is well-described (Van der Sman, 2017). Often, a small-valued interaction parameter between the two carbohydrates was required.

FH is extended towards the glassy state by Vrentas and Vrentas (1991), which is shown to hold for biopolymers (Hancock & Zograf, 1993), and numerous food systems (Van der Sman & Meinders, 2011; van der Sman, 2012). Often, other molecular thermodynamics theories hold only in the liquid state (Fredenslund et al., 1975). For the class of carbohydrates and polysaccharides, the interaction parameter is shown to be a function of the molecular weight (Van der Sman & Meinders, 2011; Van der Sman, 2017). Expanding this class to more general non-food polyols shows that the interaction parameter is a function of chain length (expressed as the number of carbons), and the ratio of the number of OH groups and carbon atoms (van der Sman, 2019b). The latter shows that it is probable that the FH interaction parameter can be related to the functional group theory. The same study shows that FH fails if the polyol has a strong non-uniform (non-random) distribution of OH-groups — like polyols with amphiphilic character. For these compounds, we expect clustering at the molecular scale, as in alcohols.

We conclude that FH theory is well able to describe the thermodynamic properties of aqueous solutions of small solutes, which interact mainly via hydrogen bonding, such as carbohydrates and amino acids. In that, it is comparable to UNIFAC and PC-SAFT. Furthermore, all three theories can describe solid-liquid equilibria for the solutes.

The predictive power of PC-SAFT and FH theories is shown for ternary mixtures comprising two solutes, but only for the case of zero interaction parameter between solutes. UNIFAC can be fully predictive for ternary or even more complex mixtures but requires thoughtful determination of the functional groups and their interaction parameters.

PC-SAFT and UNIFAC can also predict activity coefficients for solutes in solvent mixtures like water/alcohol — while there is no FH model available for that. We think the latter is due to the restricted degrees of freedom of the FH theory: there is only a single interaction.

For aqueous mixtures with small food-borne solutes, we think FH theory is only suitable to strongly hydrophilic solutes — having a uniform/random distribution of hydrogen bonding functional groups. The sweet amino-acids investigated in this paper are an example of that. FH theory will have more difficulty with amino-acids with relatively large aliphatic chains, fatty acids, or dicarboxylic acids. The value of FH theory is even stronger in complex mixtures of small hydrophilic solutes with biopolymers or oligomers, as found in a wide variety of foods, like bakery, dairy, and confectionery products — making it a good vehicle for investigating questions like sugar replacement in foods.

5. Conclusions

Taking inspiration from the novel field of NADES we have investigated whether zwitterions like amino acids and some quaternary ammonium compounds can be used as alternative sugar replacers. As sugar replacers, they should have good water sorption properties and good plasticization of biopolymers. These properties have been investigated using literature data, and data from our lab. The acquired data have been analysed with theories that have been used priorly for the analysis of polyols as sugar replacers.

Our results show that the investigated zwitterions are good plasticizers and humectants, albeit with poor solubility, with L-proline being an exception showing good solubility up to 60%–70%. Their thermodynamics can indeed be described with the same theories as previously used for common sugar replacers, namely Flory–Huggins (FH) theory and Couchman–Karasz for the glass transition. Comparing FH theory with other molecular thermodynamics theory like PC-SAFT and UNIFAC, we conclude that FH will be more suitable for strongly hydrophilic zwitterions, but it will probably fail for zwitterions with large aliphatic segments.

The poor solubility of many of the zwitterions makes it difficult to measure the glass transition. We have shown the glass transition can either be determined via a) the glass transitions of frozen solutions, which also contain better soluble carbohydrates like sucrose, or b) via extrapolating the viscosity. The viscosity of zwitterion solutions is shown to be a function of T_g/T , following the master curve earlier formulated for polyols.

Due to the poor solubility, the viscosity can only be measured for a limited range, leading to large confidence intervals for the estimated glass transition of the pure solute $T_{g,s}$. We think it is worthwhile to further investigate how $T_{g,s}$ depends on the number and type of functional groups to provide a better estimate of $T_{g,s}$ of particular amino acid-like plasticizer.

Finally, we have tested the influence of glycine and L-proline on the melting of biopolymers, namely the denaturation of egg white and gelatinization of starch. As found for carbohydrates these amino acids also depress the melting point of biopolymers, as a function of the hydrogen bond density. The effective hydrogen bond density can be computed from the glass transition of the applied solution, similar to other sugar replacers. Interestingly, we have observed indications of phase separations at quite high moisture contents, for both amino acids as common carbohydrates.

Summarizing, (sweet) amino acids and related quaternary ammonium zwitterions have good potential as sugar replacers, showing very similar behaviour as carbohydrates regarding plasticizing and humectant behaviour. Many of the amino acids have poor solubility, but that can be circumvented by using a mixture of plasticizers, which have improved solubility.

CRedit authorship contribution statement

R.G.M. van der Sman: Conceptualization, Supervision, Writing. **I.A.F. van den Hoek:** Investigation, Data curation. **S. Renzetti:** Methodology.

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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Appendix A. Supplementary data

Supplementary material related to this article can be found online at <https://doi.org/10.1016/j.foodhyd.2020.106113>.

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