Linear and non-linear rheological properties of water–ethanol hybrid pectin gels for aroma enhancement

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ABSTRACT

Whereas water–ethanol hybrid gels present an opportunity to realize aroma enhancement, translating hypothesis into practice is limited by poorly defined viscoelastic characteristics of those gels. In this work, the linear and non-linear rheological properties of water–ethanol hybrid pectin gels (WEPGs) were studied. Those WEPGs are physical gels in nature and the WEPG of 28.6% v/v ethanol differs basically from those of higher ethanol concentrations in the gel strength, resistance to deformation and non-linear properties. The retention of isopentyl acetate of WEPGs is dramatically improved by increasing the ethanol concentration to 33.3% v/v in the co-solvent system, but it is not further improved at 37.5% v/v. The cluster analysis reveals strong positive correlations between the isopentyl acetate release concentration and v_3/v_1 and absolute value of S/T ratio under 100% strain, suggesting the non-linear rheological responses of WEPGs have to be taken into account for which the enhancement of aroma is desired.

1. Introduction

Pectin is a natural multifunctional hydrocolloid with remarkable emulsifying, thickening and gelling capacities and widely used in jams, jellies, juices and beverages (Grassino, Barba, Brnčić, Lorenzo, Lucini, & Brnčić, 2018). In terms of the gelation of low methoxyl pectin (degree of methoxylation < 50%), some divalent metal ions, usually Ca^{2+}, are required to form junction zones that are constituted by binding of carboxyl groups in pectin molecules with Ca^{2+}. The formation of such a junction zone is generally described by the "egg-box model". While a low pH (<3.6) together with the presence of large amounts of co-solutes like sugar (>55%) are necessary for the irreversible gelation of high methoxyl pectin (degree of methoxylation > 50%) known as an acid sucrose gelation (Ishwarya & Nisha, 2021). Normally, monovalent cations such as Na^+ and K^+ are not expected to induce the gelation of pectin, but neutralize the negative charges of polymer chains due to the charge shielding effect (Wang, Wan, Chen, Guo, Liu, & Pan, 2019), giving rise to the intermolecular aggregation and formation of dense gel network (Ström, Schuster, & Goh, 2014).

Despite the most impactful gelling methods, pectin has been found to form water-ethanol hybrid pectin gels (WEPGs) in presence of a high concentration of ethanol (Tkalec, Knez, & Novak, 2015). Owing to the presence of the polar hydroxyl group, alcohols are more water-soluble than simple hydrocarbons and can be used as anti-solvents, which directly initiate the rapid precipitation of the water-soluble polymers and modulate the sol–gel equilibria (Jiang, Qi, Liao, & Yang, 2021; Tong et al., 2022). Besides the exclusion of cosolvent from polymer surfaces, the enhancement of water structure around the polymers induced by cosolvent can easily alter the gel structure and its rheological properties (Shimizu & Matubayasi, 2014; Zhang, Song, Tian, Zhao, & Zhou, 2022). Taking advantage of such a hybrid solvent system, we have tuned the overall rheological responses of gels by changing the alcohol concentration and regulated the distribution of small molecules as well (Zhou et al., 2020).

Another advantage of WEPGs is to overcome the challenge of enhancing the smell of pectin gels, because most volatile aroma compounds are hardly incorporated and retained in highly hydrophilic hydrogels, but can be easily dissolved in alcohols (Zhang, Song, Tian, Zhao, & Zhou, 2022) and synchronously trapped in WEPGs. As desired, the aroma-enhanced WEPGs should retain those aroma compounds when subject to food processing, such as extrusion, mixing and stirring, where large stress or strain are applied, and efficiently release them when undergoing situations like chewing and swallowing. However, the changes in the structural rearrangement of a gel could substantially

affect the entrapment, migration and diffusion of aroma compounds, and any effort to enhance retention of aroma compounds is likely to undermine their release (Su et al., 2021). Designing WEPGs for enhancing aroma without compromising the latter requires optimization of their rheological properties (Han, Xu, Dong, Wu, Wang, & Chen, 2014).

To fabricate the WEPGs for aroma enhancement, we characterized their rheological properties upon a wide range of applied strains to establish the basic rheological property database. An aroma release simulation study along with the cluster analysis was then conducted to correlate rheology related factors and the aroma release amount. In this study, the aroma release curve, the maximum intensity of aroma release and time to maximum intensity, although important, were not measured because of limited experimental instrument.

2. Materials and methods

2.1. Materials

High methoxyl apple pectin (APA104; degree of methoxylation 62.4%) was provided by Yantai Andre Pectin Co Ltd. (Shandong, China). The contents of pectin, protein, ash and moisture were determined as 88.5 ± 1.04 g/100 g, 0.3 ± 0.01 g/100 g, 2.0 ± 0.01 g/100 g and 9.2 ± 0.02 g/100 g, respectively. Other chemicals were of analytical grade and supplied by Kelong Chemical Reagent Co., Ltd (Sichuan, China).

2.2. Preparation of aroma-enhanced WEPGs

Pectin was thoroughly suspended in deionized water at 25°C to achieve a 1.5% w/v pectin dispersion. 0.2% w/v NaCl was added to the suspension to promote the gelation. The pH of the suspension was adjusted to 2.9 using 1 M HCl. According to the results of preliminary experiment, different amounts of isopentyl acetate were dissolved in specific volumes of ethanol, and then were mixed together under intense agitation with the pectin dispersions to reach a final ethanol volume fraction of 28.6%, 33.3% and 37.5% with constant isopentyl acetate concentration (0.01 g/100 g) in the mixture. The degassing process was performed by heating the sample from 25°C to 60°C and then cooling to 25°C to induce gelation. The WEPGs with 28.6%, 33.3% and 37.5% v/v ethanol were denoted as $E_{28.6}$, $E_{33.3}$ and $E_{37.5}$, respectively.

2.3. Rheological measurements

Rheological measurements were performed on a rotational rheometer (Anton Paar MCR 302, Austria) equipped with a rotational cylinder (CC27/P1, length 40.01 mm, diameter 26.66 mm). The gels were formed *in situ* in the cylinder followed by an equilibration at 25°C for 2 h before subsequent measurements.

2.3.1. Small amplitude oscillatory shear (SAOS) measurements

The frequency sweep was conducted at 25°C in the range from 0.05 to 10 Hz under a constant strain of 0.05% within the linear viscoelastic region (LVR). The traces of storage modulus (G') and loss modulus (G'') were recorded. The complex modulus (G^*) was calculated by the following equation:

$$G^* = \left(G'^2 + G''^2 \right)^{1/2} \tag{1}$$

2.3.2. Large amplitude oscillatory shear (LAOS) measurements

LAOS measurement, in a strain-controlled sweep manner, was performed by logarithmically increasing strain amplitude (γ_0) from 0.01% to 100% at 25°C and 1 Hz. The traces of both moduli as a function of γ_0 were obtained. With these traces, the archetype of non-linear behavior as well as the transition γ point (γ_t) between linear and non-linear viscoelastic regions were identified as previously reported (Hyun, Kim, Ahn, & Lee, 2002).

Within the applied strain range from 0.63 to 63%, the non-linear response based on the intensity ratios I_n/I_1 (I_n is the intensity of the *n*-harmonic signal) was characterized via the following Fourier series:

$$\sigma(t; \omega, \gamma_0) = \gamma_0 \sum_{n\ odd}^{n=1} \left\{ G'_n (\omega, \gamma_0)\sin n\omega t + G''_n (\omega, \gamma_0)\cos n\omega t \right\} \tag{2}$$

where σ is the stress, ω is the oscillation frequency, t is the time, G'_n and G''_n refer to the G' and G'' of nth-harmonic (Ewoldt, Hosoi, & McKinley, 2008), $\omega = 0.63\%$, 1.58%, 3.97%, 9.98%, 25.1% and 63% were selected.

The elastic (e_n) and viscous (v_n) Chebyshev coefficients of each harmonic can be obtained via the equation (3) and equation (4):

$$e_n = G'_n (-1)^{(n-1)/2} \tag{3}$$

$$v_n = G''_n / \omega \tag{4}$$

Typically, harmonic Chebyshev coefficients (e_3 and v_3) are used as pattern indicators in sorting the non-linear viscoelastic behaviors. Accordingly, the non-linear viscoelastic behavior can be categorized into four categories, *i.e.*, strain-softening ($e_3 < 0$), strain-stiffening ($e_3 > 0$), shear-thinning ($v_3 < 0$) and shear-thickening ($v_3 > 0$) (Hyun et al., 2011).

The strain-stiffening ratio (S) and shear-thickening ratio (T) of gels can be obtained via the equations:

$$S = \left(G'_L - G'_M \right) / G'_L \tag{5}$$

$$T = \left(\eta'_L - \eta'_M \right) / \eta'_L \tag{6}$$

Herein, G'_L is the instantaneous large-strain modulus or the secant modulus at maximum strain and G'_M is the instantaneous minimum-strain modulus or the tangent modulus at zero strain ($\gamma = 0$), while η'_L and η'_M are the instantaneous viscosities at the largest and smallest ($\dot{\gamma} = 0$) strain-rate, respectively. The obtained values could be used to consolidate the rheological patterns resulted from Chebyshev coefficients via the following rules: strain-softening ($S < 0$), strain-stiffening ($S > 0$), shear-thinning ($T < 0$) and shear-thickening ($T > 0$) (Ewoldt, Hosoi, & McKinley, 2008).

2.4. Determination of aroma release upon simulative mastication

For the aroma release analysis, 4.0 g WEPG was formed *in situ* in a sealed glass vial (22.5 mm i.d.) and equilibrated at 25°C for 2 h. Then WEPG was compressed and punctured with a glass cylindrical plunger (with a diameter of 10 mm) for 1 min, which made up-and-down (approximate 52 cycles min^{-1}) movements to imitate the breakdown of gel during chewing (Hansson, Leufvén, & van Ruth, 2003). Vials were sealed immediately after the breakdown of WEPG to prevent the escape of isopentyl acetate.

Then release of isopentyl acetate were evaluated according to the method reported by Fan et al. (2018) with some modifications. In brief, the sealed gel-loaded vial was incubated at 37°C for 30 min. Isopentyl acetate in the headspace of the vial was extracted by a solid-phase microextraction needle (50/30-μm, DVB/CAR on PDMS; Supelco, Inc., PA, USA) at 37°C for 30 min. After that, the needle was immediately inserted into the inlet (250°C) of a gas chromatography-mass spectrometry (Trace MS/GC; Thermo Quest Finnigan Co., CA, USA) and held for 5 min. To start the analysis, helium as the carrier gas was supplied at a flow rate of 0.8 mL/min in a splitless mode. A TG-5MS elastic quartz capillary column (30 m × 0.25 mm i.d.) was equipped. The initial column temperature was 40°C and kept for 3.0 min, and then raised to 60°C by 2°C/min and held for 2 min, then further increased to 230°C by 5°C/min, and finally held at 230°C for 15 min.

Considering the possible variation of isopentyl acetate concentration in the gelling and degassing process, the isopentyl acetate
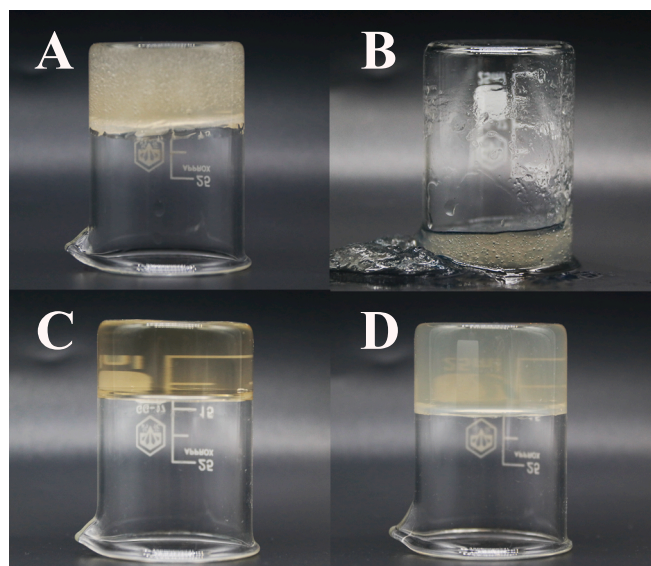
Fig. 1. Appearances of the WEPG formed upon intense agitation (A), the pre-gel of WEPG formed without intense agitation (B), the acid sucrose pectin gel (C), and the degassed WEPG (D).

concentrations before and after gelling and degassing were analyzed for each gel and results signified no significant difference. To represent an accurate measure of the aroma release extent, the concentration of isopentyl acetate was presented as the peak intensity in arbitrary unit (Weel et al., 2002) and individually normalized by the initial concentration of isopentyl acetate before breakdown.

2.5. Statistical analysis

All measurements were conducted at least in triplicate. One-way analysis of variance (ANOVA) using Duncan's multiple range test for evaluating the differences among groups with the SPSS statistics software (version 23.0). Significant differences among groups were indicated at $p < 0.05$.

Python package Seaborn was performed for creating the cluster heatmap based on the property database. Linear correlation coefficients were computed for each pair of factors. Seaborn cluster map function was adopted, which utilized the Euclidean distance metrics to generate a

linkage matrix for hierarchical clustering (Oliveira, Fasolin, Vicente, Fuciños, & Pastrana, 2020).

3. Results and discussion

3.1. Key points of WEPGs preparation

The preliminary study indicated the sol–gel transition of pectin in water–ethanol mixture was dependent on the ethanol concentration and the critical gelation concentration of ethanol was determined to be 26.7% v/v. Thus, ethanol concentrations higher than that critical concentration were adopted for ease of handling in fabricating WEPGs for aroma enhancement.

In practice, intense agitation in the mixing process of ethanol and pectin solutions was demonstrated to be crucial to the prevention of pre-gel formation associated with the deterioration of solvent properties and resultant localized irreversible aggregation (Zhang, Song, Tian, Zhao, & Zhou, 2022). Despite a more efficient mixing of ethanol and water, intense agitation could prohibit locally preferential competitive hydration between the pectin molecule and ethanol, which might alter the thermodynamics and of the given system, resulting in marked changes in the sol–gel equilibria (Shimizu & Matubayasi, 2014). Fig. 1A showed a typical WEPG formed with the aid of intense agitation. In contrast, the formation of mesogel phase and subsequent phase separation was observed without intense agitating during mixing (Fig. 1B).

Obviously, more air bubbles can be found in WEPGs in the comparison with traditional pectin sugar acid gels (Fig. 1C), as a consequence of the air inhalation during agitation and its imprisonment upon gelling. In fact, rheological studies on gels were sensitive to the presence of such bubbles in a manner consistent with experimental observations. Thus, a degassing process was required in this study. Among all the attempted methods, simply heating to 60°C proved its effectiveness in degassing and was implemented in following studies (Fig. 1D).

3.2. Linear rheological properties of WEPGs

The mechanical spectra of WEPGs were illustrated in Fig. 2. The G' trace of each sample stayed above its G'' trace regardless of frequency. In Fig. 2, the gel with a higher ethanol concentration always presented higher values in G', G'' and corresponding $G*$, which was sensitive to reflect the overall gel strength (Yang, Nisar, Liang, Hou, Sun, & Guo, 2018). A higher $G*$ value was normally attributed to the higher cross-linking density involved in the strong gels (Saavedra Isusi, Karbstein,
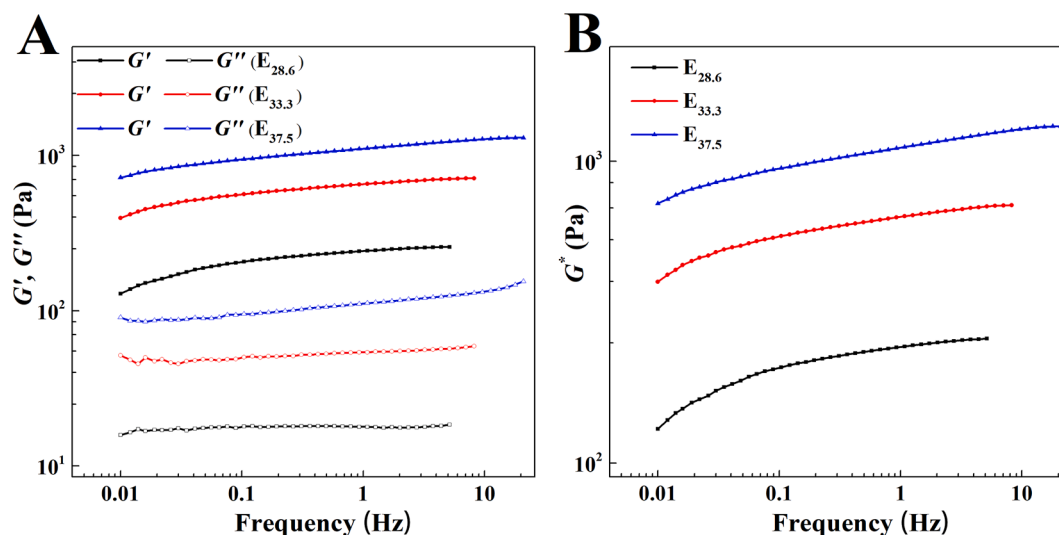
Fig. 2. Frequency sweep of WEPGs at various ethanol concentrations: 28.6% v/v ($E_{28.6}$), 33.3% v/v ($E_{33.3}$) and 37.5% v/v ($E_{37.5}$). Frequency-dependence of storage modulus (G', closed symbols), loss modulus (G'', open symbols) (A) and complex modulus ($G*$) (B).
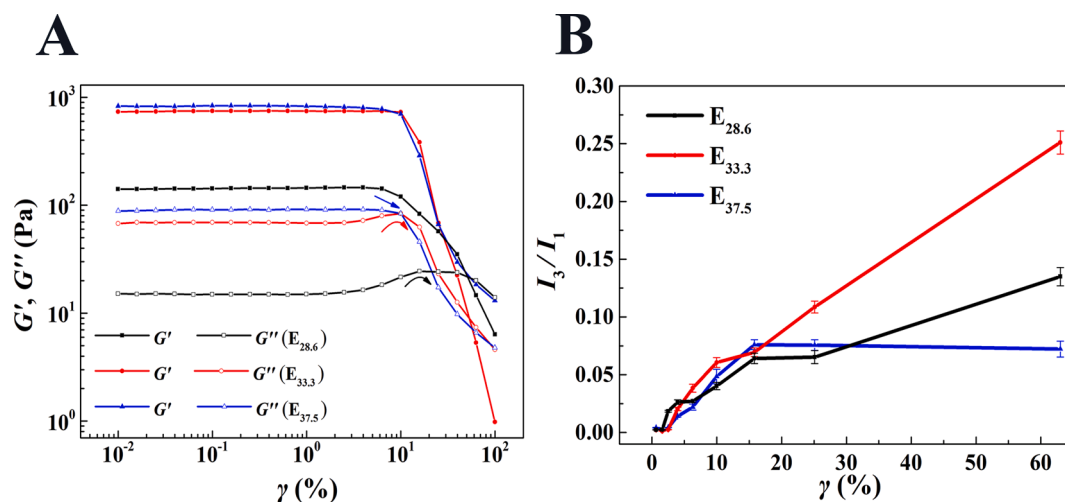
Fig. 3. Amplitude sweep of WEPGs at various ethanol concentrations: 28.6% v/v ($E_{28.6}$), 33.3% v/v ($E_{33.3}$) and 37.5% v/v ($E_{37.5}$). Strain-dependence of storage modulus (G', closed symbols) and loss modulus (G'', open symbols) at 1.0 Hz (A); The I_3/I_1 intensity ratio as a function of strain amplitude γ% (B).

& van der Schaaf, 2019). However, it should be noted that each WEPG showed mechanical spectra not identical to those strong gels: the profile of both moduli showed an obvious frequency dependence with no sign of a Newtonian plateau in the low frequency range with G' exceeding G'' at all frequencies. Such behavior is intermediate in properties between the two extremes by entanglement networks and cross-linked networks. For highly branched polysaccharide like pectin, despite the convincing evidence for junction zone associated in the form of "egg cage" (Cao, Lu, Mata, Nishinari, & Fang, 2020), no speculation has been made to date on the variation in conformation of polymer chain and hence this behavior probably does not stem from the conformational transition.

A notable difference in dynamic moduli between gels of 28.6%, 33.3% and 37.5% v/v ethanol was apparent. Except for the concentration of ethanol, the concentration of pectin in WEPGs was slightly different, specifically 1.07%, 1.00% and 0.94% w/v for $E_{28.6}$, $E_{33.3}$ and $E_{37.5}$. Usually, the strength of gels decreases on polymer chain dilution (Andlinger, Schlemmer, Jung, Schroeter, Smirnova, & Kulozik, 2022). However, dynamic moduli of WEPGs seemed to be only relevant to the concentration of ethanol, illustrating the chain association involved by ethanol dominated the linear rheological behaviors in the case of WEPGs.

As is known, frequency represents the time scale in reality. In this context, all samples were gel at given time-scale range of 0.01 s − 1 s, but the frequency-dependent behavior also demonstrated their weak gel nature (Sherahi, Shadaei, Ghobadi, Zhandari, Rastgou, & Hashemi, 2018). As the above considerations suggest, we can reasonably assume that different pathways or extents of chain association result in three-dimensional networks of WEPGs with various levels of connectivity and junction zones of disparate length and strength, which in turn, accounts for the existence within the class of weak gels, characterized by more (*e.g.*, $E_{28.6}$) or less (*e.g.*, $E_{37.5}$) pronounced frequency dependence and the differences in the absolute value of the G', G'' and G^*.

### 3.3. Non-linear rheological behaviors of WEPGs

Frequency sweep has been demonstrated to be useful to reveal the gel viscoelasticity in the non-destructive state, but such a setup could not go deep into the intended processing of interest, where the existence of irreversible changes of gel structure is also consistent with non-linear response (Sobanwa, Foster, Yakubov, & Watson, 2022). In the case of weak gels, we can easily extend dynamic measurements to large deformations without rupture of the gel or formation of macroscopic discontinuities inside the WEPGs.

3.3.1. Leading order description of non-linear viscoelasticity

The leading-order LAOS behaviors were obtained from the inspection of the strain amplitude sweep of the present gels (Fig. 3A). The limit of the LVR was determined as 2.51%, 3.97% and 3.97% for $E_{28.6}$, $E_{33.3}$ and $E_{37.5}$ gels, respectively, beyond which progressive transition from linear to non-linear rheological response took place (Anvari & Joyner, 2018). This critical deformation characterizes the sensitivity of a given system that sustains deformation to maintain its integrity (John, Ray, Aswal, Deshpande, & Varughese, 2019) and enables a distinction to be made between entangled solutions and gels. As a general rule, the LVR of the latter may extend to larger strains of the order unit compared to that of the former. Obviously, these critical deformations were close to each other, once again signifying the similar weak gel character of WEPGs.

As depicted in Fig. 3A, both moduli of $E_{37.5}$ decreased sharply with strain in the non-linear viscoelastic region, a typical strain thinning LAOS behavior. The origin of strain thinning was thought to be related with the destruction of network junctions (Hyun et al., 2011) and the homogeneous flow as a consequence. However, in the non-linear viscoelastic region, the trend of G'' for $E_{28.6}$ and $E_{33.3}$ was distinguished from that of $E_{37.5}$. There was a visible peak on their G'' curves beyond the linear strain limit for $E_{33.3}$ and $E_{28.6}$ and such an overshoot in G'' has been referred to as a type III response or the Payne effect, which is a continuum of transient behaviors typically associated with plasticity and yielding in soft materials (Goudoulas & Germann, 2018; Hyun, Kim, Ahn, & Lee, 2002). The G'' overshoot in soft materials with a yield transition has been elucidated as the transition from primarily solid-like, viscoelastic dissipation in the linear regime to primarily fluid-like, plastic flow at larger amplitudes by a time-resolved experimental strain decomposition. As is well known that the existence of a yield transition distinguishes weak gels from polymer solutions and melts and, conversely, underlines the contiguity with highly concentrated suspensions. The transition was initiated as the elastic solid-like component decreases, while the plastic component begins to slow its increase, peaking slightly after the G'' does. At large amplitudes, both components decrease, although the dissipation is still primarily due to unrecoverable plastic flow. The decrease of G' can be explained as the introduction of a significant unrecoverable strain component results in a reduced percentage of the total strain amplitude being from the recoverable strain, necessarily reducing the normalized elastic potential energy storage (Donley, Singh, Shetty, & Rogers, 2020). In a gel system, a spatially heterogeneous transition arising from the transient destruction and reformation of the network could account for the changes in elastic solid-like and plastic components. We found the slope of this increase of G'' varies in samples, being steeper for $E_{33.3}$ and shallower for $E_{28.6}$,
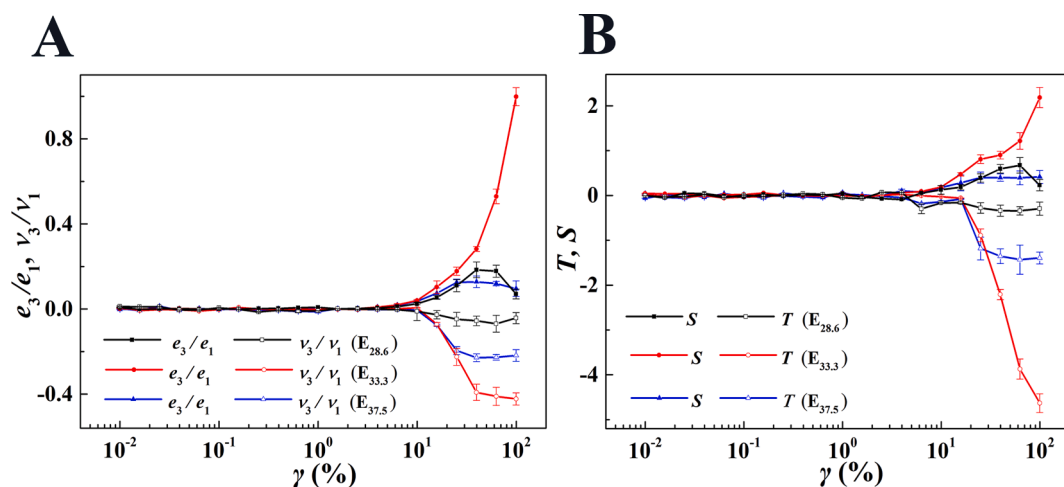
Fig. 4. Quantitative analysis of non-linear response of WEPGs at various ethanol concentrations: 28.6% v/v ($E_{28.6}$), 33.3% v/v ($E_{33.3}$) and 37.5% v/v ($E_{37.5}$). The normalized 3rd elastic (e_3/e_1, closed symbols) and viscous (ν_3/ν_1, open symbols) Chebyshev coefficients as the function of strain amplitude (A); The strain-stiffening ratio (S, closed symbols) and shear-thickening ratio (T, open symbols) as the function of strain amplitudes (B).

suggesting a substantial modification in the network connectivity and homogeneity by ethanol (Donley, Singh, Shetty, & Rogers, 2020).

3.3.2. Degree of non-linearity

Indeed, soft matters with diverse non-linear viscoelasticity could display the same leading-order LAOS type behavior and distinguishing the leading-order LAOS type only based on first-harmonic viscoelastic moduli was insufficient and/or misleading for describing their non-linear rheological behaviors. It was therefore of importance to analyze these differences in distortions (higher harmonics) of stress waveform under LAOS (Ma, Su, Wang, Li, & Wang, 2020).

The presence of higher harmonic was considered a sign of deviation from linear to non-linear behavior (Alvarez-Ramirez, Carrera-Tarela, Carrillo-Navas, Vernon-Carter, & Garcia-Diaz, 2019). The signal intensity normalization against that of the first harmonic (fundamental frequency) was introduced to the analysis of the harmonic components (Hyun et al., 2011). Among all the I_n/I_1 upon amplitude, I_3/I_1 was normally the most intense one and thus was chosen for quantitively measuring the non-linearity of WEPGs. As we can see from Fig. 3B, the I_3/I_1 values of WEPGs were higher than 0.05, a significant intensity assumed for non-linearity. $E_{33.3}$ was significantly higher in I_3/I_1, indicating $E_{33.3}$ was superior in non-linearity and the degree of non-linearity were concluded in the order of $E_{33.3} > E_{28.6} > E_{37.5}$. It should be noted that non-linear spectroscopy was very sensitive to structural details and a gel with a higher non-linearity would be expected to respond differently in a complex manner with increasing strain amplitude (Kim, Song, Choi, & Hyun, 2019). In this sense, amplitude sweep in focusing on non-linear rheological behaviors of WEPGs helped to distinguish between the case of breakdown of the original gel network into small cluster regions with a higher level of heterogeneity ($E_{33.3}$ and $E_{28.6}$) and the case where the network junctions almost completely disappear with flow properties typical of a disperse system ($E_{37.5}$). We noted that the increased elastic solid-like properties of WEPGs could be achieved with ethanol concentration increasing, but not necessarily implied a progressive breakdown of networks in the non-linear viscoelastic region.

3.3.3. Quantitative analysis of non-linear rheological response

Chebyshev polynomials were used as orthonormal basis functions to further decompose each of those stresses into harmonic representations, e_n and ν_n. The Chebyshev coefficients of third harmonic were, in essence, treated as the major parameters of non-linearity and hence the coefficients normalized by the first one (e_3/e_1 and ν_3/ν_1) were derived as shown in Fig. 4A. As expected, e_3/e_1 and ν_3/ν_1 were identically zero in the linear domain, suggesting only the first harmonic contributed to the

viscoelastic behaviors and the rheological response of WEPGs could be completely described by the leading order coefficient (Tong, Xiao, Cheng, Chen, & Sun, 2018). However, when approaching the non-linear region, a strain-stiffening elastic response of WEPGs was indicated generically by a positive third order elastic Chebyshev coefficient, $e_3/e_1 > 0$, whereas shear-thinning was indicated by a negative viscous Chebyshev coefficient $\nu_3/\nu_1 < 0$ (Ewoldt, Hosoi, & McKinley, 2008). The magnitude of e_3/e_1 or ν_3/ν_1 could be used to verify the quantitative measure of the degree of non-linearity and it could be concluded that the shear-thinning effect of WEPGs was in the order of $E_{33.3} > E_{37.5} > E_{28.6}$, while the corresponding strain-stiffening effect order was determined as $E_{33.3} > E_{28.6} > /\approx E_{37.5}$.

In principle, the parameters of e_3/e_1 and S presented similar changing trends against the deformation amplitude, and so did the parameters of ν_3/ν_1 and T (Hyun et al., 2011). But importantly, the S-T reconstruction of the response was valuable to provide a better description of the actual overall non-linear response (Joyner, 2021). The non-linear characteristics for each gel as implied in Fig. 4B were supported by the positive S and the negative T. Moreover, for $E_{33.3}$ and $E_{37.5}$, their absolute values of T were much higher than those of S, indicating their overall shear-thinning behaviors upon large deformation (Precha-Atsawanan, Uttapap, & Sagis, 2018). Such gels display a partially elastic-partially viscous behavior and give thinner consistencies during swallowing, which are potential to be used in foods designed for deglutition difficulties (Lorenzo, Zaritzky, & Califano, 2013).

Alcohols (e.g., methanol, ethanol and glycerin) have manifested themselves as gelation promoters in various studies with underlying mechanism being explained by either the excluded volume effect which renders those gelling polymers shrinking at low hydration levels or affecting the structural interactions: water molecules tend to be more restrained around those small molecules, promoting polymer–polymer interactions rather than polymer–solvent interactions (Sun et al., 2021; Tong et al., 2022). As a result, aggregation into super strands makes up a stiff but irregular network structure. However, from a theoretical point of view, there is not much fundamental work being carried out to explain the non-linear rheological behaviors based on sophisticated network characterizations and to establish a quantitative structure-ethanol concentration relationship for WEPGs (Zhang, Song, Tian, Zhao, & Zhou, 2022).

3.4. Aroma release from WEPGs

The volatiles differed in chemical structures such as functional groups (e.g., aldehydes, ketones, esters, alcohols), resulting in varying
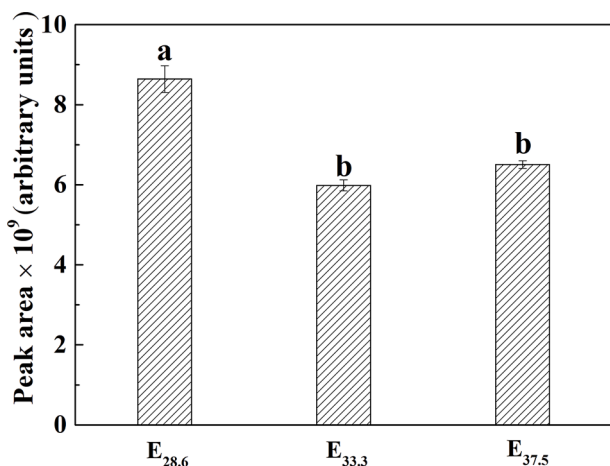
Fig. 5. The release amount of isopentyl acetate from WEPGs at various ethanol concentrations: 28.6% v/v ($E_{28.6}$), 33.3% v/v ($E_{33.3}$) and 37.5% v/v ($E_{37.5}$).

hydrophobicity. The conclusions of major investigations indicate esters are the most volatile among systematic series and increasing chain length leads to increased binding within homologous series. Isopentyl acetate is naturally produced by ripening fruit and widely used to improve the flavor and smell of foods. But the high partition coefficient $K_{air/water}$ of isopentyl acetate suggests the extent of its retention in gels needs to be facilitated (Zafeiropoulou, Evageliou, Gardeli, Yanniotis, & Komaitis, 2012). Thus, aroma release of WEPGs was investigated using isopentyl acetate as a model aromatic compound by semi-quantification of isopentyl acetate in the headspace of vials containing WEPGs.

As shown in Fig. 5, the release concentration of isopentyl acetate from $E_{28.6}$ was significantly higher than that from both $E_{33.3}$ and $E_{37.5}$. There are two aspects to consider, to begin with the concentration variation of ethanol, which is a versatile solvent encouraging isopentyl acetate to distribute through the hydrophilic matrix. But the trend was that the headspace aroma concentration decreased coincidently with the ethanol concentration increasing to 33.3%, which, however, tends to stay constant, when subsequently elevating the ethanol concentration to 37.5%. That implied other factors may exert an influence on the aroma release from gels. On that topic, Bylaite, Ilgūnaitė, Meyer, and Adler-Nissen (2004) reported that aroma compounds with high $K_{air/water}$ were most affected by the presence of biopolymers in the matrix, probably due to weak interactions between highly hydrophobic compounds and the biopolymer chain and during gel network breakdown, their release would be substantially affected.

In other words, the network structure was bridging the mechanical property and the aroma release of a gel. A previous study clearly showed that a higher concentration of aroma compounds in the headspace could be detected in softer gels. The aroma compound may interact with the hydrophobic parts of the pectin chains more intensively in softer gels due to a looser gel structure (Tyapkova, Bader-Mittermaier, Schweigert-Weisz, Wurzinger, Beauchamp, & Buettner, 2014). In a comparison between $E_{33.3}$ and $E_{37.5}$, both of which had higher gel strength than $E_{28.6}$, the released amount was not significantly different. As discussed in detail in section 3.3.3, the non-linear responses of those gels were quite different in the balance of viscous and elastic contributions and the viscously-dominated non-linear behavior was assigned to only $E_{33.3}$ and $E_{37.5}$. This brings us the hypothesis that the overall non-linear response plays a core role in influencing aroma release.

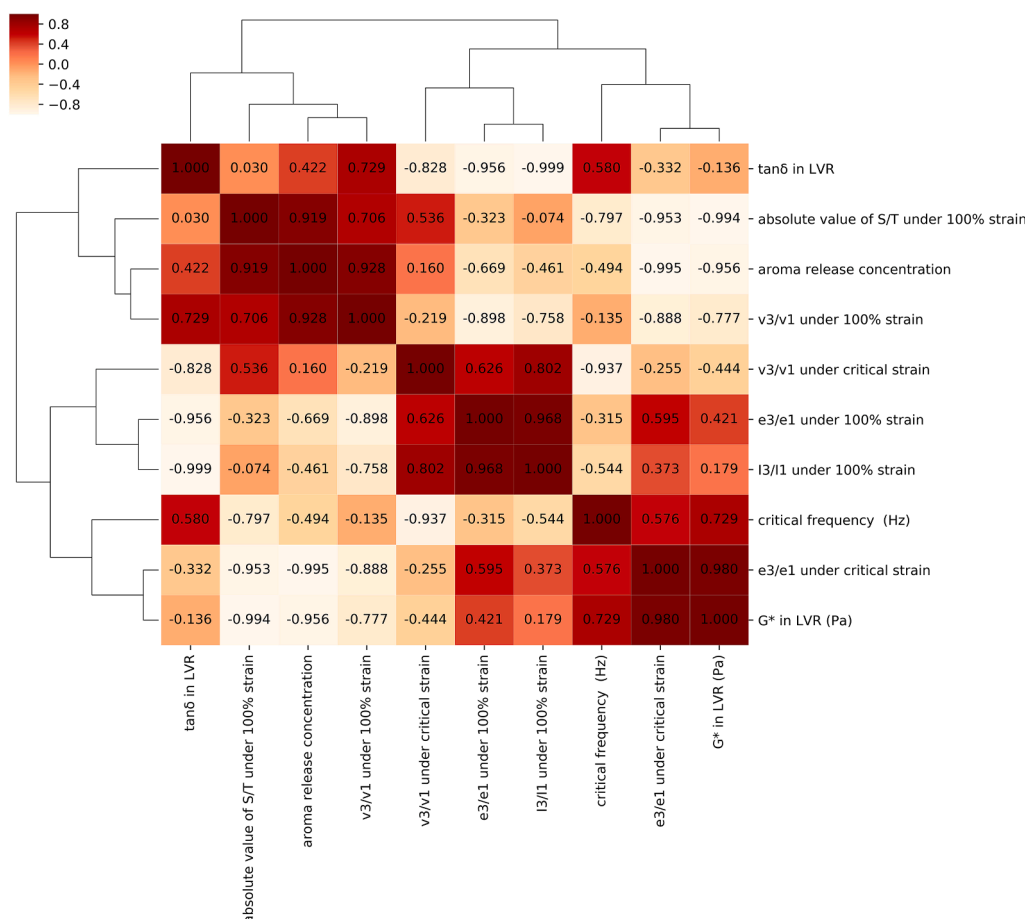This hypothesis gave rise to the question which rheological indicator

Fig. 6. The cluster heatmap of the typical rheological indicators and the aroma release concentration of WEPGs with visualized correlation coefficients and networks.

was the determining one promising for controlling or predicting the aroma release. Herein, the correlation factors between rheological indicators and aroma release concentration of WEPGs were determined by clustering to figure out the multi-factor correlation. LAOS indicators included I_3/I_1, ν_3/ν_1, e_3/e_1, absolute value of S/T ratio under 100% strain and ν_3/ν_1, e_3/e_1 under critical strain, while average $\tan\delta$ and $G*$ in LVR were used as the SAOS indicators. As independent indicators, the critical frequency was also included in establishing the correlativity. Some indicators such as G', G'' in LVR and I_3/I_1, S/T at critical strain had been excluded because they were either meaningless or highly collinear with those indicators aforementioned or meaningless.

Those correlations were shown in Fig. 6. There were several very strong correlations being recognized, *e.g.*, $G*$ in LVR (−0.956), e_3/e_1 under critical strain (−0.995), ν_3/ν_1 under 100% strain (0.928) and absolute value of S/T ratio under 100% strain (0.919). It could be inferred from the results that not just the gel strength, but also the elastic and viscous contributions of non-linear viscoelasticity governed the aroma release of WEPGs. The strain-stiffening under critical strain could be even more relevant and important than the gel strength in regions of low applied stress when predicting the aroma release from a gel which had been reformed in the oral processing.

4. Conclusions

There has been a consensus that the release of aroma from gel is a complex phenomenon, depending on both the volatility of the aroma compounds and the mechanical properties of gels. However, the category of gels constituted with water–ethanol co-solvents collects those systems whose solvent properties are intermediate between those typical of hydrogels and those peculiar to organogels, rendering the release of aroma even more difficult to predict.

In the case of WEPGs, the enhanced retention of aroma compounds could be clearly evidenced with a higher concentration of ethanol, but the maneuver of aroma releasing through solely increasing the ethanol portion may lead to questionable results. A thorough rheological discrimination among all the WEPGs indicated both linear and non-linear rheological behaviors of WEPGs could be dramatically changed with varying ethanol concentrations. Some non-linear rheological indicators such as the ν_3/ν_1 and absolute value of S/T ratio under 100% strain were strongly correlating the aroma release concentration, demonstrating the non-linear viscoelasticity probably governed or at least perturbed the aroma release of WEPGs. Accordingly, the non-linear rheological response of WEPGs has to be taken into account when aroma enhancement is the objective of designing WEPGs.

This study could thus lead researchers to combine models or modify them to account for the very complex nature of aroma entrapment and release in a gel system and offer the additional benefit of relating aroma release to some simple and directional rheological indicators.

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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