Impact of ternary NADES prepared from proline, glucose and water on the Maillard reaction: Reaction activity, Amadori compound yield, and taste-enhancing ability

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ABSTRACT

This study employed proline, glucose, and water to prepare natural deep eutectic solvents (NADES) through heating and stirring. The Maillard reaction was then performed, producing a high yield of Amadori rearrangement product (ARP) and physicochemical properties of NADES were examined for impacts on the reaction. Water had a dual function by promoting the formation of hydrogen bonding networks within the NADES when present at less than 15%, and also working as a diluting agent that could potentially disturb its structure when exceed 15%. These changes further affected the subsequent Maillard reaction, especially the ARP accumulation (reached the highest when water content was 15%). Correlation analysis shows strong positive viscosity-ARP and negative water activity-ARP correlations within a range. Moreover, the product (rich in ARP) remarkably enhanced umami and saltiness. This finding provides insights into modulating the Maillard reaction by adjusting NADES properties, demonstrating feasibility of this approach for flavor enhancer development.

Introduction

The Maillard reaction refers to a series of complex chemical reactions that occur between carbonyl compounds (such as reducing sugars) and amino compounds (which include amino acids, peptides, and proteins) (Ogasawara et al., 2006; Zhang et al., 2019). The reaction can be divided into three stages: the early stage, the advanced stage, and the final stage (Zhang et al., 2018). The UV absorbance at 294 nm and 420 nm are frequently employed to assess the content of intermediates and browning products, providing a general evaluation of the development of the Maillard reaction (Li et al., 2022). In addition, comprehensively understanding the properties of key Maillard reaction products (MRPs), such as Amadori rearrangement products (ARPs), is vital for investigating and applying the Maillard reaction.

ARPs predominantly form in the initial stage of the Maillard reaction, where N-glycosylamine undergoes a spontaneous Amadori rearrangement reaction, resulting in the formation of the corresponding 1-amino-1-deoxy-2-ketose (Cui et al., 2021). ARPs are important in food flavor research because they are precursors of various volatile compounds. Additionally, they can also significantly enhance the umami and/or salty tastes., making food taste better with less need for monosodium glutamate (MSG) and nucleotides (Zhang et al., 2022). The poor thermal stability of ARPs leads to their easy decomposition into advanced MRPs, thus resulting in low formation yields. This has limited the utilization of these compounds. Therefore, a "food-grade" Maillard mimetic preparation method was developed to efficiently produce ARPs by combining lyophilization and thermal treatment, which takes advantage of the potential of natural deep eutectic solvents (NADES) to influence the Maillard reaction (Zhang et al., 2020). This method, using food-grade components and simple processing steps, has the potential to prepare ARP in a manner suitable for food-grade applications.

NADES can be identified as a subcategory of deep eutectic solvents (DES), which were first proposed to describe the mixture of urea and choline chloride (Abbott et al., 2003). Both urea and choline chloride are solid starting materials characterized by high melting points. When they mixed, the resulting eutectic system demonstrates an extensive liquid

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range and possesses fascinating properties that make it well-suited for various solvent applications (Dai et al., 2013; Smith et al., 2014). NADES are DES derived from natural compounds (like sugars, sugar alcohols, amino acids, organic acids, and organic bases), with promising applications as green solvents (Dai et al., 2013; Smith et al., 2014; Zhang et al., 2012). Given the potential of NADES as efficient solvents and their ability to influence the Maillard reaction, as evidenced by their applications in catalysis, organic synthesis, and extraction processes, it is essential to explore their impact on ARP yield.

Proline has the highest solubility among amino acids and is one of the most common natural ingredients capable of forming NADES (Dai et al., 2013). It was also indicated that proline-derived ARPs possess excellent umami-enhancing and saltiness-enhancing properties (Wang et al., 2021). Thus, different ternary samples composed of proline, glucose, and water were constructed via heating-stirring method (some are NADES), which were then subjected to physicochemical property detection (water activity, viscosity, differential scanning calorimetry (DSC) and Fourier-transform infrared spectroscopy (FTIR)) and Maillard reaction. This study aims to investigate the effects of NADES properties on Maillard reaction activity and examine their correlation with the yield of ARPs, as well as the taste-enhancing ability of proline-glucose ARP, thereby establishing a comprehensive understanding of the relationship between NADES, Maillard reaction, and taste enhancement.

Material and methods

Materials and chemicals

Deionized water was prepared in the laboratory using a Milli-Q Gradient system (Millipore, Schwalbach, Germany). Other reagents were purchased from the following manufacturers: L-proline and D-glucose (analytical grade, >99 %) from Genview Corporation, L-proline and D-glucose (food grade, 97 %–98 %) from Star Lake Bioscience Co, Inc. Zhaoqing Guangdong, hydrochloric acid and sodium hydroxide (analytical grade, >99 %) from Guangzhou Chemical Reagent Factory, monosodium glutamate (food grade, 98 %) from Henan Lotus Flavor Co., Ltd., sodium chloride (food grade, 99 %) from Guangdong Salt Industry Group Guangzhou Co., Ltd., methanol (chromatographic grade, >99.8 %) from Thermo Fisher Scientific, and formic acid (chromatographic grade, >99.8 %) from Sigma-Aldrich Corporation.

Sample formulation

After mixing proline and glucose in different molar ratios and adding water (pH 7.0) at varying contents (w/w), the mixture was subjected to heating and stirring at 50 °C for 90 min to produce a heat-stirred mixture comprising proline, glucose, and water (HS). Each sample was named based on the preparation method, molar ratio of proline and glucose, and water content. For instance, place 1.15 g of proline and 1.80 g of glucose (in a molar ratio of 1:1) into a glass bottle, then add 15 % distilled water (pH 7.0). After sealing the bottle, position it in a 50 °C water bath and heat with magnetic stirring for 90 min. The resulting sample is labeled as HS-1:1–15 %. Similarly, when the pH values of added water range from 5.0 to 9.0, the resulting samples are respectively labeled as HS-1:1-pH5, HS-1:1-pH6, HS-1:1-pH7, HS-1:1-pH8, and HS-1:1-pH9. A separate sample, named FD-1:1, was obtained by dissolving equimolar amounts of proline and glucose in a 90 % water solution (pH 7.0) and subsequently subjecting the mixture to freeze-drying. Alternatively, when the same mixture was dissolved in a 90 % water solution (pH 7.0) without undergoing freeze-drying, it resulted in the formation of the sample: aqueous solution-1:1.

Infrared spectroscopic analysis

The experiments were conducted using the reported method (Xin et al., 2017), with slight modifications. The spectra of samples were performed by the FTIR (Thermo Fisher Scientific, Waltham, Massachusetts, USA). FTIR spectra were collected from 400 to 4000 cm^{-1} at room temperature (resolution of 4 cm^{-1}).

Thermodynamic analysis

DSC measurements were conducted using a DSC 214 Polymer device (NETZSCH-Gerätebau GmbH, Karlsruhe, Germany). The temperature program consisted of an initial cooling step from 20 to −60 °C at a rate of 10 °C/min, followed by a subsequent heating step from −60 to 20 °C at a rate of 10 °C/min.

Determination of viscosity and water activity

Viscosity measurements were conducted using a Brookfield viscometer (Ametek Inc., Bethlehem, USA). The sample was transferred into a 50 mL centrifuge tube, and a volume of 25 mL was used for viscosity testing at room temperature. Water activity was determined using an Aqualab 4TE moisture activity meter (METER Group, Inc., Pullman, USA). A volume of 5 mL of the sample was used for the measurement.

Determination of Maillard reaction activity

The samples were respectively heated at 80 °C for 30, 60, 90, 120, 180, and 240 min, followed by immediate storage at −25 °C. After the reaction, the samples were diluted with distilled water to a concentration of 0.2 mg/mL (based on the initial glucose concentration before the reaction). The formation of Maillard reaction intermediates (MRIs) was monitored using a UV–visible spectrophotometer (Shanghai Metash Instruments Co., Ltd., Shanghai, China) by measuring the absorbance at 294 nm, while the formation of brown polymers was monitored by measuring the absorbance at 420 nm.

The analysis of ARP made from proline and glucose using UPLC-MS/MS

The content of ARP during the reaction was detected using the Q Exactive™ Hybrid Quadrupole-Orbitrap™ Mass Spectrometer (Thermo Fisher Scientific, Waltham, USA) by calculating the peak area. Chromatographic separation was achieved using a Hypersil GOLD C18 column (2.1 × 100 mm, 3.0 μm, Thermo Fisher Scientific, Waltham, USA). The UPLC conditions included an injection volume of 1 μL, a concentration of 2 μg/mL, a flow rate of 0.3 mL/min, mobile phase A consisting of 0.1 % formic acid water, and mobile phase B consisting of methanol. The gradient elution conditions were as follows: 0–1 min, 2 % B; 1–1.5 min, 2–95 % B; 1.5–5.4 min, 95 % B; 5.4–6 min, 95–2 % B; 6–10 min, 2 % B, with no program heating. The mass spectrometer was operated with the following conditions: electrospray ionization source, positive ion mode, Full-MS + MS2 acquisition mode, scanning range of 70–1000 *m/z*, and collision energy of 15 eV. The structure of the target ARP has been determined through comprehensive preliminary study (Zhang et al., 2020), involving the synthesis and purification of the compound, followed by its structural characterization using mass spectrometry and nuclear magnetic resonance techniques (Please refer to Supplementary data for more details of methods).

Sensory evaluation

Sensory analysis was performed following the reported method in a sensory evaluation room with constant temperature of (25 ± 2)°C and 70 % humidity (Zhang et al., 2023). A sensory panel comprising 12 individuals, aged between 25 and 35, was established for this study. All panel members had prior sensory experience of at least one year and underwent a comprehensive one-week training program (3 times) primarily utilizing the triangle test and rating scale method to enhance their judgment of umami and salty taste, as well as their perception of taste intensity. This training ensured their proficiency in sensory

Table 1
Composition and physical properties of proline and glucose mixtures made by heating and stirring.

Name	Composition	Molar ratio	Water content	pH	Appearance	Water activity	Viscosity (Pa·s)	Equivalent solubility[a]	
								Proline 162 g[b]	Glucose 91 g[b]
HS-5:1–15 %	Proline:Glucose	5:1	15 %	7.0	Transparent, liquid, some crystals	–	–	432	135
HS-4:1–15 %	Proline:Glucose	4:1	15 %	7.0	Transparent, liquid, some crystals	–	–	407	159
HS-3:1–15 %	Proline:Glucose	3:1	15 %	7.0	Transparent, viscous, liquid	0.556	1.237	372	194
HS-2:1–15 %	Proline:Glucose	2:1	15 %	7.0	Transparent, viscous, liquid	0.563	1.214	318	249
HS-1:1–15 %	Proline:Glucose	1:1	15 %	7.0	Transparent, viscous, liquid	0.579	1.193	221	346
HS-1:2–15 %	Proline:Glucose	1:2	15 %	7.0	Transparent, liquid, some crystals	–	–	137	429
HS-1:3–15 %	Proline:Glucose	1:3	15 %	7.0	White, solid–liquid mix	–	–	100	467
HS-1:4–15 %	Proline:Glucose	1:4	15 %	7.0	White, solid–liquid mix	–	–	78	489
HS-1:5–15 %	Proline:Glucose	1:5	15 %	7.0	White, solid–liquid mix	–	–	64	502
HS-1:1–0 %	Proline:Glucose	1:1	0 %	7.0	White, solid–liquid mix	–	–	–	–
HS-1:1–5 %	Proline:Glucose	1:1	5 %	7.0	Transparent, viscous, liquid	0.434	24.052	741	1159
HS-1:1–10 %	Proline:Glucose	1:1	10 %	7.0	Transparent, viscous, liquid	0.498	6.166	351	549
HS-1:1–20 %	Proline:Glucose	1:1	20 %	7.0	Transparent, viscous, liquid	0.627	0.405	156	244
HS-1:1–25 %	Proline:Glucose	1:1	25 %	7.0	Transparent, viscous, liquid	0.682	0.238	117	183
HS-1:1–50 %	Proline:Glucose	1:1	50 %	7.0	Transparent, dilute solution	0.868	0.048	39	61
HS-1:1-pH5	Proline:Glucose	1:1	15 %	5.0	Transparent, viscous, liquid	0.579	1.193	221	346
HS-1:1-pH6	Proline:Glucose	1:1	15 %	6.0	Transparent, viscous, liquid	0.579	1.193	221	346
HS-1:1-pH8	Proline:Glucose	1:1	15 %	8.0	Transparent, viscous, liquid	0.579	1.193	221	346
HS-1:1-pH9	Proline:Glucose	1:1	15 %	9.0	Transparent, viscous, liquid	0.579	1.193	221	346

[a] By evaluating the proline/glucose and water content in each sample, the proline/glucose concentration in the sample can be calculated relative to the water content. This concentration is then expressed as the amount of proline/glucose dissolved in a 100 mL aqueous solution (25 °C).

[b] The value represents the maximum solubility of proline or glucose in water (100 mL, 25 °C). The data is sourced from The National Center for Biotechnology Information.

evaluation techniques. Prior to their participation, each panel member willingly agreed to take part in the study by signing informed consent forms. The recruitment and consent process strictly adhered to the guidelines outlined in the Declaration of Helsinki, ensuring ethical considerations were met. Panelists tasted 2 mL of each sample, kept it in the mouth for 30 s before expectorating. They were then asked to rate the intensity of umami taste. To avoid fatigue and carryover effects, panelists were instructed to rinse their mouths with 50–60 mL of drinking water between samples. A 0.05 % MSG solution was prepared by dissolving MSG in water as the umami base liquid, while a 0.1 % sodium chloride (NaCl) solution was prepared by dissolving NaCl in water as the salty base liquid. The prepared samples (0.25 mg/mL) were added to the base liquids, and the control group consisted of base liquids without the samples. Subsequently, panelists used a rating scale ranging from 0 to 10 (0: no taste, 3: 0.05 % MSG solution/0.1 % NaCl solution, 10: 0.075 % MSG solution/0.15 % NaCl solution) to assess the umami or salty taste of the sample solutions. The intensity of the sample's umami/ salty taste was then categorized into four levels: "-" denoting no enhancement (0–3 points), "+" representing average enhancement ability (3–5 points), "++" indicating stronger than average enhancement ability (5–7 points), and "+++" signifying significantly stronger than average enhancement ability (7–10 points).

Statistical analysis

Statistical significance was evaluated using analysis of variance (ANOVA) performed in triplicate with SPSS 26.0 software (SPSS Inc., Chicago, IL, USA). Tukey's HSD test was applied as the post-hoc comparison when the ANOVA indicated significant differences among groups. Additionally, the software was utilized for typical analyses and Pearson correlation analyses.

Results and discussion

Preparation of NADES via heating and stirring

Table 1 describes the composition of proline and glucose mixtures and their physical properties after heating and stirring treatment. It could be observed that different proportions of proline and glucose, as well as varying water content, result in distinct appearances of the mixtures. NADES are usually transparent liquids with a certain viscosity, or may be in semi-solid states (Liu et al., 2018). The majority of samples listed in Table 1 demonstrate a transparent and viscous liquid state. Nonetheless, certain samples lack stability and display the presence of crystals. Furthermore, there exist samples that manifest as turbid and viscous solids. These findings are consistent with prior studies, indicating that while proline and glucose are common components of NADES, NADES can only be formed under suitable proportional conditions (Dai et al., 2013). Claire R. Ashworth's research corroborates the aforementioned phenomenon. Specifically, in the DES comprising choline chloride and urea, the formation of the hydrogen bond network can occur through two distinct ionic pair structures, namely ChCl-U and ChCl-Cl-U, both coexisting within the solvent. Modifying the component ratio has the potential to modify the mode of combination, consequently influencing the formation of the hydrogen bond network (Ashworth et al., 2016). Likewise, the proportion of water plays a significant role in the formation and stability of NADES (Liu et al., 2018).

Furthermore, Table 1 specifically presents the proportions of proline and glucose in the mixture relative to the water content in each mixture (expressed as the mass of each component per 100 g of water). Clearly, in all transparent, viscous liquid mixtures, the calculated concentration of proline or glucose is markedly higher than its corresponding maximum solubility in water (in some cases both are higher than their respective maximum solubilities). The HS-1:1–50 % sample stands as the only exception, with the proline and glucose concentrations falling below their respective maximum solubilities in water, thus the solutes could fully dissolve in water, presenting a transparent, dilute aqueous solution state, rather than the state of other samples. This represents a characteristic feature of NADES, whereby the concentrations of its constituent components are typically significantly higher than their maximum solubilities in aqueous solutions. This unique characteristic of NADES sparked the hypothesis that they could function as a third liquid phase in living organisms, providing an alternative to water and lipids, and play a pivotal role in diverse biological functions (Choi et al., 2011; Yuliana et al., 2011). Hence, it is hypothesized that samples displaying a transparent and viscous liquid state are typical NADES, whereas samples showing a transparent appearance with some crystals are a combination of NADES and solid components. Conversely, white solid–liquid
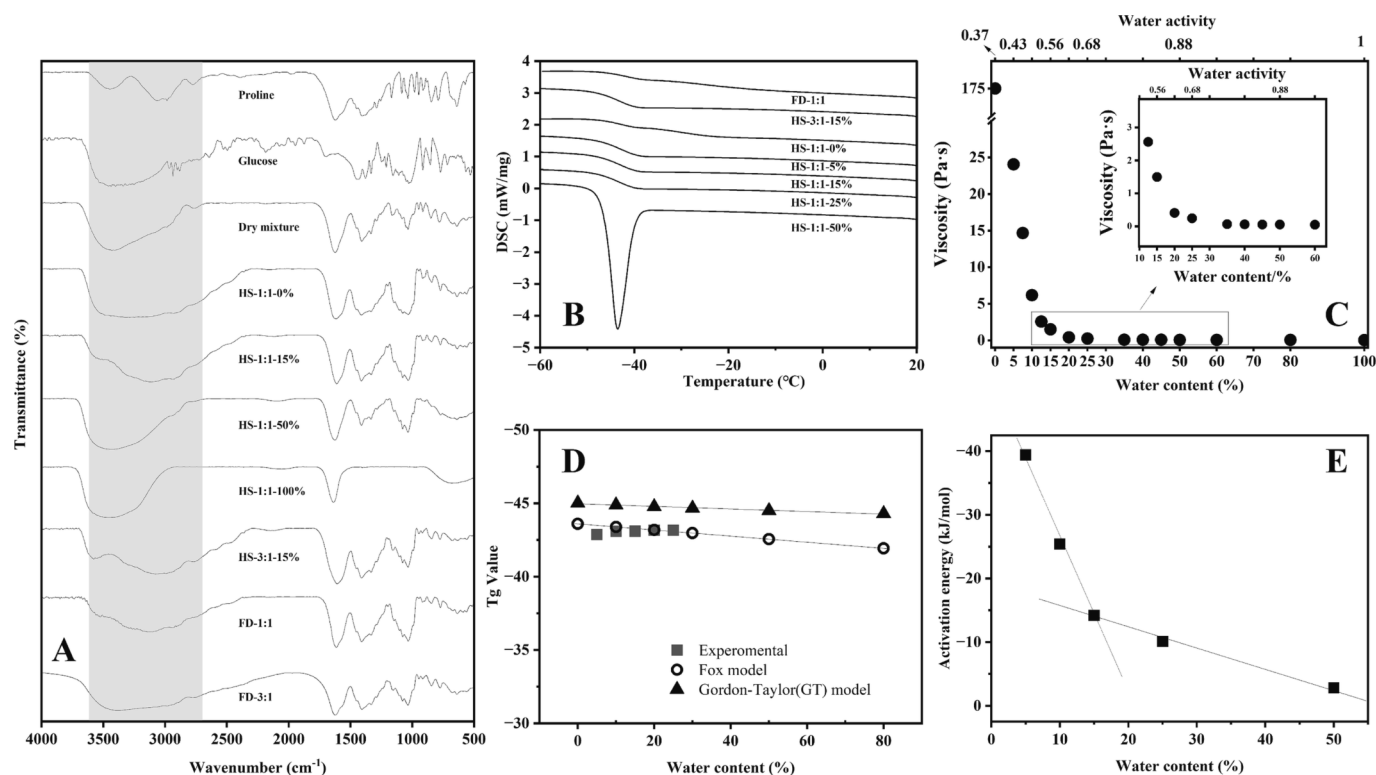
Fig. 1. Physicochemical properties of proline-glucose mixtures prepared by heating and stirring with different compositions. **A:** Fourier transform infrared (FTIR) spectrum; **B:** Differential scanning calorimetry (DSC) thermograms; **C:** Relationships between water content, water activity, and viscosity; **D:** Evolution of measured glass transition temperature (Tg) values and simulated Tg values obtained by different model methods as a function of water content; **E:** Activation energy of the samples as a function of water content.

mixtures and transparent dilute solutions are not regarded as NADES.

Properties of the heat-stirred mixtures consisting of proline, glucose, and water

The formation of NADES is attributed to the intermolecular interactions, among which, hydrogen bonds play the most crucial role as the primary driving force (Liu et al., 2018). FTIR spectrometry and 1H NMR spectroscopy have been widely used to recognize the hydrogen bond-induced modifications by comparing them to the individual isolated components (Dai et al., 2013; Liu et al., 2018; Smith et al., 2014). As the high viscosity of some NADES samples may lower the resolution of 1H NMR spectra, FTIR was selected in this research. In addition, distinguishing different NADES or NADES from aqueous/solid phase samples can be accomplished through the analysis of various physicochemical properties, including the phase transition behavior, viscosity, water activity, and other material properties (Liu et al., 2018).

Fig. 1A provides evidence of intermolecular interactions between proline and glucose used in NADES. The spectra exhibit interesting features, particularly the interaction observed in proline-glucose-water mixtures. The NADES samples (HS-1:1–15 %, HS-3:1–15 %, FD-1:1, FD-3:1) formed by proline and glucose displayed a prominent flat-broad band (3600–2500 cm^{-1}) in contrast to the spectra of the starting materials (proline and glucose). This band is attributed to the stretching and bending vibrations of –OH groups, indicating the generation of extensive hydrogen bonds between proline and glucose (Jangir et al., 2020; Ren et al., 2018). The downward shift of the –OH group absorption band from 2500 to 2000 cm^{-1} provided additional confirmation of hydrogen bond formation between the initial compounds (Santana et al., 2019). The absorption band of the proline NH group shifted from 1625 to 1608 cm^{-1}, indicating the involvement of glucose and proline in hydrogen bond (O—H···N—H) formation. The bending

absorption band of –OH groups in water molecules within NADES was observed at 1623–1608 cm^{-1} (Santana et al., 2019). Importantly, changes were also observed in the fingerprint region (Ren et al., 2018). Additionally, the FTIR spectra of NADES formed by proline and glucose at various molar ratios (1:1, 3:1) revealed a shift in the broad –OH band from 2986 to 2994 cm^{-1}. The increase in water content from 15 % to 100 % resulted in a red-shift of the hydroxyl absorption band and the disappearance of flat-broad band (3600–2500 cm^{-1}), indicating the disruption of hydrogen bonds in NADES (Cao et al., 2021).

The thermal behavior of those samples were assessed through DSC, aiming to evaluate the impact of water content on the material properties of mixtures. Fig. 1B describes that samples with water content below 50 % solely displayed the glass transition without any other thermal events within the experimental temperature range. The glass transition temperature (Tg) of these samples ranged from −44 to −42 °C, significantly lower than the melting points of proline (228 °C) and glucose (146 °C). This finding suggested that the mixtures exhibited amorphous behavior without any indication of water crystals (Ren et al., 2018). This observation confirmed the formation of NADES (Castro et al., 2018). In contrast, for the sample with a water content of 50 %, no glass transition region was identified in the DSC thermogram. Instead, an exothermic peak corresponding to melting was observed. These findings suggested that the sample displayed thermal behavior similar to an aqueous solution, indicating the lack of a supramolecular structure with abundant hydrogen bonds. This observation is in accordance with the infrared results depicted in Fig. 1A, thus corroborating the speculative conclusions presented in Section 3.1.

Viscosity and water activity are common physical properties that are primarily influenced by the chemical characteristics of sample components and intermolecular interactions (Altamash et al., 2018; Chemat et al., 2015). Viscosity represents the capacity of a fluid to resist flow induced by an applied force. It also indicates the strength of
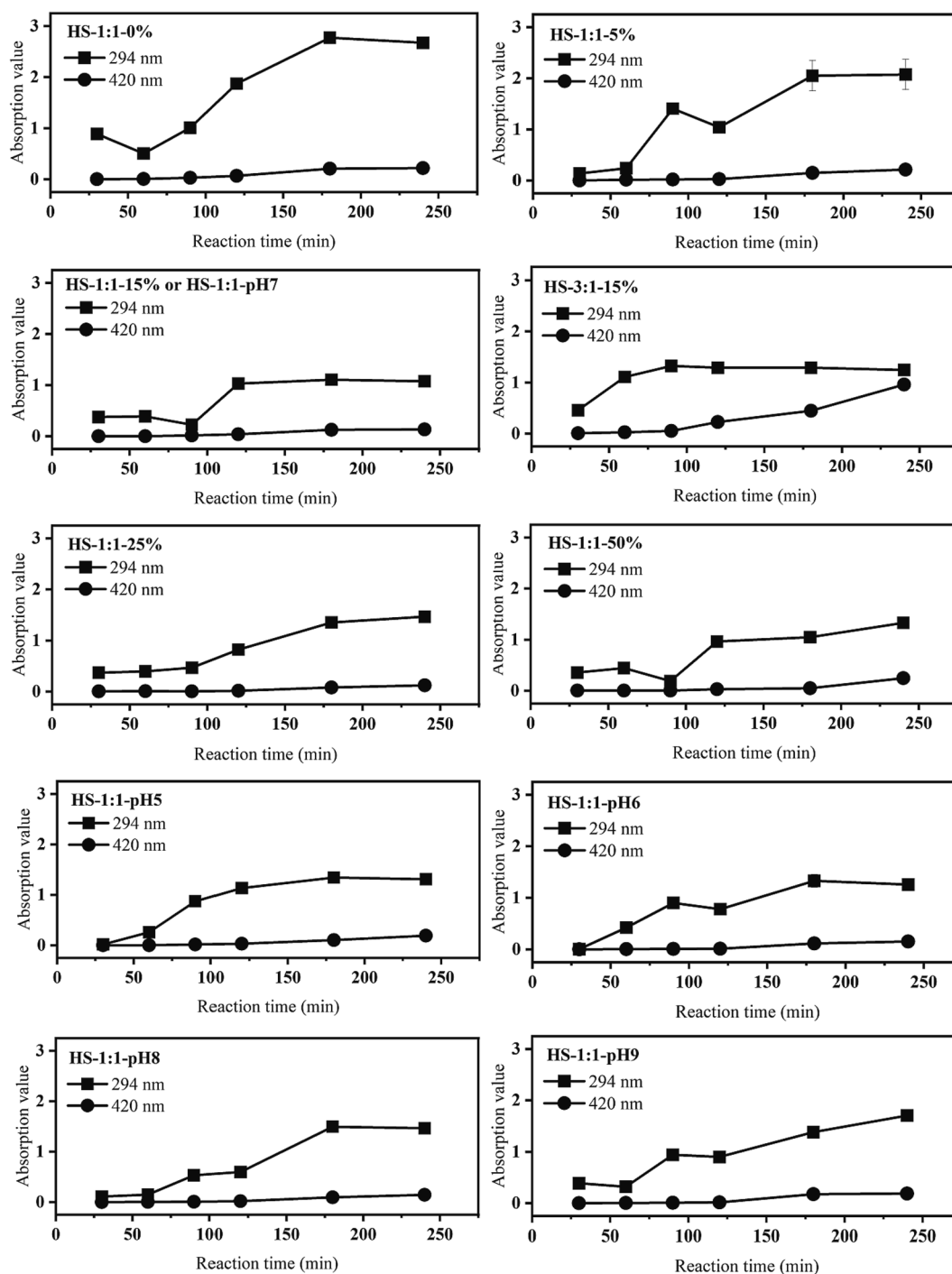
Fig. 2. Time-dependent absorbance of Maillard reaction products at 294 nm and 420 nm in proline-glucose mixtures prepared by heating and stirring. Error bars are included but may not be visible due to small measurement variability.

intermolecular interactions between the components in the fluid, which correlates with the number and bonding patterns of hydrogen bonds (Caprin et al., 2021). Fig. 1C depicts the relationship among water activity, viscosity, and water content of the samples. Based on the water activity and water content, the samples could be roughly categorized into three types: aw = 0–0.45 (water content 0–5 %, ultra-low moisture samples), aw = 0.45–0.8 (water content 5–50 %, low moisture samples), and aw = 0.8–1 (water content 50–100 %, high moisture samples) (Caprin et al., 2021). As aw increased from 0 to 0.45, the sample's viscosity exhibited a rapid decrease from an almost immeasurable value of 175 Pa·s to 24.052 Pa·s. During this stage, the sample predominantly

consists of solid materials, including proline, glucose, and certain NADES. As aw increased from 0.45 to 0.8, the sample's viscosity continued to decrease from 24.052 Pa·s to 1.193 Pa·s. During this stage, proline, glucose, and water achieved complete dissolution, accompanied by the formation of numerous hydrogen bonds. These interactions contributed to the establishment of a stable NADES structure with reduced viscosity. When aw > 0.8, the sample's viscosity experienced a gradual decrease to 0.048 Pa·s. This decrease was attributed to the dilution caused by the presence of excessive water, which enhanced ion dissociation and hydration effects. Consequently, the stable supramolecular structure of NADES progressively disintegrates, resulting in a

transition toward a conventional aqueous phase state.

The experimentally obtained Tg values for the NADES samples (samples with water content more than 50 % did not have correct Tg values) were compared with the values calculated using the Fox and Gordon-Taylor models (Fig. 1D). The Fox model is commonly employed to predict Tg in classical homogeneous mixtures consisting of components with similar solubility that display weak interactions (Caprin et al., 2021; Fox, 1952). In contrast, the Gordon-Taylor (G-T) model is primarily used to predict Tg values in binary systems, such as sugar-water mixtures (Gordon & Taylor, 1952). Fig. 1D shows that the experimental Tg values were consistently lower than the ones predicted by the G-T model and closer to the Tg values predicted by the Fox model, irrespective of the molar composition of the mixture. These differences suggested weak interactions among the components in the samples.

Flow activation energy (Ea) refers to the energy necessary for molecules to overcome the frictional forces exerted by neighboring molecules and initiate movement at a specific temperature. Since the sample predominantly adheres to the Arrhenius law, it is possible to deduce the flow activation energy (Ea) (Caprin et al., 2021). Fig. 1E presents the flow activation energies (Ea) of prepared mixture samples with water content ranging from 5 wt% to 50 wt%. The Ea values were higher for mixtures with lower water content and decreased significantly with increasing water content. This trend suggested that the solvents exhibit cohesive behavior, attributed to the formation of abundant and temperature-dependent hydrogen bonds among proline, glucose, and water (Aroso et al., 2017; Savi et al., 2019). Interestingly, the plot of Ea against water weight content showed two separate linear regions with differing slopes, intersecting at a water content of 15 wt%. This intersection indicated the presence of two phases characterized by different degrees of the water effect: i) a phase with a steep slope where water actively contributed to NADES formation by participating in the hydrogen-bond network, and ii) a second phase where water primarily acted as a diluting molecule (Caprin et al., 2021). The results clearly demonstrated that the water content has a significant impact on the physical properties of ternary NADES composed of proline, glucose, and water. Water could participate in the formation of the supramolecular structure as a component and also act as a solvent for polar glucose and proline molecules, potentially leading to significant changes in the internal structure of NADES. These findings may help relate the molecular-level interactions to the micro and macro characteristics observed. What impact do these changes have on the subsequent Maillard reaction?

The Maillard reaction activity of the heat-stirred mixtures consisting of proline, glucose, and water

In food research, UV absorbance values at 294 nm and 420 nm are widely employed to evaluate changes in intermediate products and the extent of browning in the Maillard reaction. Despite its limited information capacity, this method is known for its stability and convenience, and it has been applied in thousands of studies, enabling cross-comparisons among diverse research works (Zhang et al., 2018; Zhang et al., 2016). Fig. 2 shows that the absorbance value at 294 nm for all samples exhibited a significant increase only after 90 min of reaction and subsequently reached a plateau. However, it is worth noting that the maximum value of the 294 nm absorbance decreased significantly when the water content of the sample exceeded 15 %. The samples HS-1:1–10 % and HS-1:1–20 % also followed the patterns described above. Additionally, the absorbance value at 420 nm of most samples exhibited minimal changes with prolonged heating time. Nevertheless, at a proline-to-glucose ratio of 3:1, the absorbance value at 420 nm exhibited a continuous increase over time, exceeding the absorbance values of other samples at equivalent time points. The experimental findings suggested that the ratio of proline to glucose exerts a greater influence on the Maillard reaction process and activity in the system compared to the water content. Moreover, an increase in the proportion of proline facilitated the accumulation of final products. This observation aligns
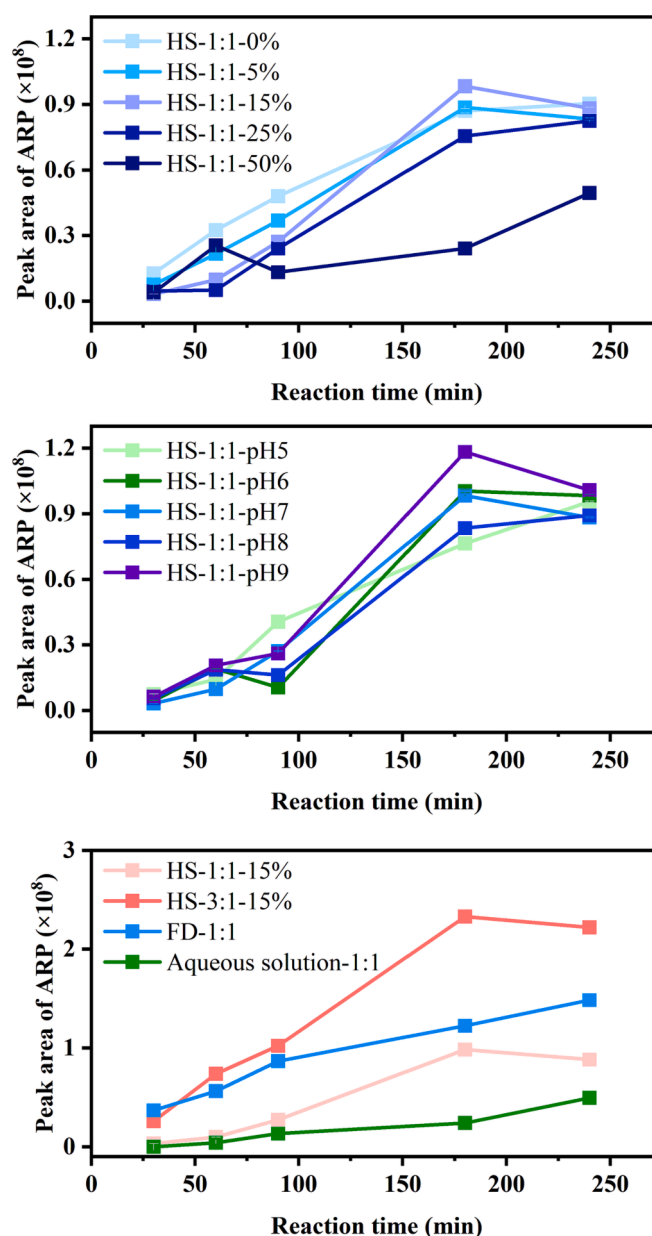

Fig. 3. Time-dependent yield of Amadori rearrangement product (ARP) from the Maillard reaction between proline and glucose in heated and stirred mixtures. Error bars are included but may not be visible due to small measurement variability.

with prior research, which highlights the significant impact of amino acid content on browning intensity and its ability to react with certain Maillard reaction products, thus promoting browning activity (Geng et al., 2019). The difference in absorbance values at 294 nm and 420 nm for NADES samples with varying pH levels was minimal, which differs from other studies (Li et al., 2022; Zhang et al., 2016). This could be attributed to the low water content of the system (15 %) and the limited impact of hydrogen ions and hydroxide ions introduced into the reaction.

The yield of ARP and correlation analysis between the properties of heat-stirred mixtures and ARP yield

Time-dependent yield of ARP during the Maillard reaction

Our previous research demonstrated an efficient preparation of ARP through the Maillard reaction based on NADES. The NADES were
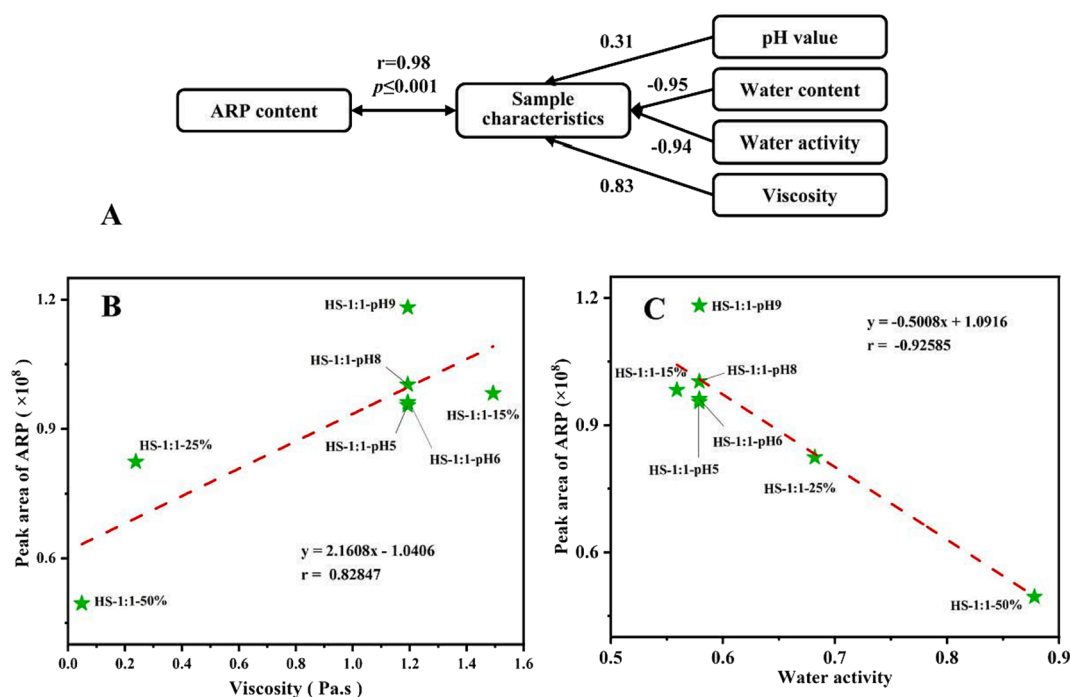
Fig. 4. Impact of physicochemical properties of heated and stirred proline-glucose mixtures on ARP yield. **A:** Typical correlation analysis between pH, water content, water activity, viscosity, and ARP yield; **B:** Pearson correlation analysis between viscosity and ARP yield; **C:** Pearson correlation analysis between water activity and ARP yield.

prepared via lyophilization (Zhang et al., 2020). As an alternative to the lyophilization method used previously, this study introduces a more efficient and industrially suitable method for ARP preparation. The Maillard reaction was conducted using NADES prepared by simple heating and stirring, in contrast to lyophilization employed before. Fig. 3 depicts the ARP yield under different reaction times. The yield exhibited an initial increase, peaking at 180 min, followed by a gradual decrease. Furthermore, excessive or insufficient water contents were unfavorable for ARP accumulation. The optimal yield of ARP was achieved when the NADES water content is 15 % under the given experimental conditions. Additionally, appropriately introducing hydroxide ions (i.e., increasing the system's pH value) could enhance the ARP yield. At last, the maximum ARP yield was compared among four methods: liquid sample, lyophilized NADES with 1:1 proline to glucose ratio, NADES with 1:1 proline to glucose ratio prepared by heating and stirring, and NADES with 3:1 proline to glucose ratio prepared by the same method. The results showed that the Maillard reaction conducted in the NADES system produces a significantly higher ARP yield compared to that in the liquid system. Moreover, preparing NADES with an excessive amount of one component, such as a 3:1 proline-to-glucose ratio, can lead to an ARP yield 2.3 times higher than freeze-dried NADES.

Correlation analysis between the properties of heat-stirred mixtures and ARP yield

The viscosity, water activity, water content, and pH value of the NADES sample are all indicators that indirectly characterize its material properties. A canonical correlation analysis was conducted to investigate the effects of these indicators on ARP production. This is depicted in Fig. 4A. The results showed a significant correlation ($r = 0.98$, $p \leq 0.001$) between NADES properties and ARP production. Notably, water content ($r = -0.95$) and water activity ($r = -0.94$) were negatively correlated with ARP yield, while viscosity ($r = 0.83$) exhibited a positive correlation with ARP yield. Subsequently, Pearson correlation analysis was conducted separately for the viscosity and water activity with ARP production, yielding correlations as high as 0.83 and 0.93 (Fig. 4B and C). These findings suggested that the NADES micro-structure had a

direct influence on the reaction. Within a certain range, ARP production was found to increase with decreasing water activity and increasing viscosity of the sample. This could be attributed to the naturally low eutectic nature of NADES, where the ionization degree of water is significantly weakened compared to aqueous systems. As a result, the decomposition of ARP is effectively prevented, thus leading to a higher accumulation of ARP during the Maillard reaction (Feather & Mossine, 2005; Geng et al., 2019).

The umami-enhancing and saltiness-enhancing abilities of the samples with different ARP content

In the end, Maillard reaction products with different ARP concentrations were prepared via freeze-drying and heating-stirring methods. Afterwards, their ability to enhance the umami/salty taste of base liquids was investigated. Table 2 demonstrates that neither proline nor glucose alone, nor their respectively corresponding heated products, enhanced the taste intensity of MSG or NaCl solutions. However, the Maillard reaction products prepared using proline-glucose based NADES, which contained high levels of ARP, exhibited varying degrees of enhancement in umami and salty taste perception. Moreover, the ability to enhance umami taste becomes stronger with increasing ARP concentrations. Meanwhile, significant enhancement of salty taste is observed only in samples with higher ARP concentrations. These results indicated that the ARP produced from proline and glucose have the potential to enhance umami and salty taste, aligning with the research findings in other studies (Wang et al., 2021). Moreover, as suggested by previous studies, it is possible that other Maillard reaction products, including aroma compounds, may also contribute to taste enhancement (Niimi et al., 2014).

Conclusion

In conclusion, the formation of NADES was feasible by mixing proline and glucose at a proper ratio, but it required a specific water content. Water content had an important impact on the internal structure of

Table 2
Taste and taste enhancing abilities of Maillard reaction products of proline and glucose.

Sample	ARP content[a]	Taste quality	Taste enhancing ability[b]	
			In the presence of MSG	In the presence of NaCl
HS-3:1–15 %[c]	6	Bland (corn aroma)	+++	+
FD-1:1[c]	3	Bland (corn aroma)	++	+
HS-1:1–50 %[c]	1	Slight sweet (slight corn aroma)	+	−
Heat-treated proline and glucose[d]	0.2	Sweet, slight bitter (slight corn aroma)	−	−
Heat-treated proline[e]	0	Sweet and bitter	−	−
Heat-treated glucose[f]	0	Sweet	−	−
Proline + Glucose[g]	0	Slight sweet and slight bitter	−	−

[a] The content of ARP made from proline and glucose in sample HS-1:1–50 % was set to 1, and the content of ARP in other samples was expressed as a multiple of the ARP content in sample HS-1:1–50 %.

[b] - no enhancement (0–3 points); + average enhancement ability (3–5 points); ++ taste enhancement ability stronger than average (5–7 points); +++ taste enhancement ability significantly stronger than average (7–10 points).

[c] The samples HS-3:1–15 %, FD-1:1, and HS-1:1–50 % were heated at 80 °C for 180 min.

[d] 1.15 g of proline and 1.8 g of glucose were dissolved in 100 mL of distilled water. The sample was then heated at 80 °C for 180 min after adjusting the pH to 7.0.

[e] 1.15 g of proline was dissolved in 100 mL of distilled water. The sample was then heated at 80 °C for 180 min after adjusting the pH to 7.0.

[f] 1.8 g of glucose was dissolved in 100 mL of distilled water. The sample was then heated at 80 °C for 180 min after adjusting the pH to 7.0.

[g] 1.15 g of proline and 1.8 g of glucose were dissolved in 100 mL of distilled water followed by adjusting the pH to 7.0.

the ternary NADES. The change in Ea suggested that water contributed positively to NADES formation by participating in the hydrogen bond network. However, when water content exceeded 15 %, the dilution effect became evident. Significant changes in NADES structure facilitated accumulation of ARP in subsequent Maillard reactions, limiting advanced products like melanoidins (excess amino acids disrupted this balance). Correlation analysis revealed a strong relationship between NADES viscosity and water activity with ARP yield within a certain range. Viscosity and water activity reflected component interactions and hydrogen bonding. This demonstrated that precise control of the Maillard reaction could be achieved by adjusting NADES structural characteristics. Our primary focus next will be determining and analyzing NADES structures precisely by integrating computational chemistry and wave function analysis, to advance investigation of chemical reaction mechanisms in NADES. This will help further increase ARP yield and enhance umami and saltiness of Maillard reaction products from proline and glucose.

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.

Data availability

The data that has been used is confidential.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2023.100905.

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