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ORIGINAL PAPER

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COMPARISON OF DIAFILTRATION-ULTRAFILTRATION AND ENZYMATIC PREPARATION OF SOY PROTEIN ISOLATE AND ITS EFFECTS ON BEVERAGE TASTE

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ABSTRACT

Background. Soy protein isolate (SPI) is frequently utilized in baked goods, meat products, fortified foods, and other applications because of its high nutritional content and great functional qualities. Although SPI's application scope and demand continue to expand, the application of SPI in the beverage industry has not been affirmed due to its properties. In this paper, SPI and 7S were extracted using double enzyme treatment (PP-SPI and PP-7S), and ultrafiltration-diafiltration (DUD-SPI and DUD-7S) were used as raw materials, respectively.

Material and methods. The functional properties and application characteristics of SPI and 7S in 'Gatorade' sports beverages, a juice beverage of freshly squeezed orange juice, and the carbonated beverage 'Sprite' were studied. The DUD-SPI and DUD-7S were added to 'Gatorade', juice, and 'Sprite' and showed low acidity, good taste, and high clarity.

Results. Electronic tongue bionic test results speculated that the addition of DUD-SPI and DUD-7S had little effect on the original flavor of 'Gatorade' and 'Sprite'. In vitro, simulated protein fluid, and saliva experiments demonstrated that the aggregation of DUD-SPI and DUD-7S was significantly reduced after mixing with saliva. The viscosity of DUD-SPI and DUD-7S mixed with saliva was slightly higher than that of the SPI and 7S.

Conclusion. As a result of their improved technical and functional qualities and pleasant mouthfeel, SPI and 7S produced by ultrafiltration-diafiltration could be used as hypoallergenic components in various acid-soluble beverages.

Keywords: ultrafiltration infiltration, soy protein isolate, taste dilution analysis, acid-soluble beverage, taste

INTRODUCTION

Soy has been widely used in processed meals for many years due to its high concentration of high-quality proteins (Herreman et al., 2020). It is incorporated in a variety of meals, including baked goods, cereals, and meat-based foods, as well as hypoallergenic infant formula and vegetarian diets, to provide certain functional features like enhanced texture, moisture retention, emulsification, and protein fortification (Day, 2013; Tan et al., 2023). However, the allergic potential of soy is one of the most significant disadvantages of soy-containing food products.

Taste is an essential issue for soy protein drinks. The most prominent problem with the taste of the currently developed acidic soy protein isolate (SPI)

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beverages is the bitter and astringent taste in the mouth after the protein is ingested, which leads to the unpleasant feeling of tightness of the tongue (Soares et al., 2017). This is caused by isoelectric precipitation of the SPI and can be avoided by adding a masking agent or reducing the molecular weight of the protein (Ding et al., 2021; Gharibzahedi and Smith, 2021). Using water-soluble polysaccharides, alkali metal salts, organic acid alkali metal salts, essential monosaccharides, and basic oligosaccharides as masking agents may reduce the stability of the SPI (Lu et al., 2019). Conversely, the molecular weight of SPI can be reduced through proteolysis (Duque-Estrada et al., 2019; Fahoum et al., 2017). However, it is crucial to select suitable hydrolysis conditions to achieve the optimal taste of the SPI, as proteolysis may increase bitterness while inhibiting astringency.

In recent years, extensive research has been conducted on the removal or hypoallergenic modification of soy protein isolate (SPI) (Ekezie et al., 2018; Wang et al., 2023; Zheng et al., 2020). Various thermal and nonthermal processing methods, including microwave, high-pressure processing, pulsed ultraviolet light, pulsed electrical fields, irradiation, high-intensity ultrasound, as well as genetic or chemical alterations have been employed to obtain SPI (Ekezie et al., 2018; Gharibzahedi et al., 2018; Pan et al., 2022). However, most of these treatments have not been successful in fully eliminating the targeted allergenic epitopes, or their efficacy has not been thoroughly investigated yet. Ultrafiltration has gained significant attention as it allows for the retention of SPI concentration while removing oligosaccharides and minerals as permeates (John and Sinha, 2019). Ultrafiltration has been utilized to obtain SPI with improved qualities and without excessive use of chemicals (John and Sinha, 2019).

The protein content of soy protein drinks is generally no less than 0.5%. Compared with animal protein, soy protein provides a more balanced nutritional profile. It not only satisfies basic thirst-quenching needs but also quickly supplements nutrition. Soy protein is an important source of protein for humans. Additionally, soy protein drinks do not cause lactose intolerance, making them suitable for the dietary preferences and habits of a wide range of consumers. Moreover, soy protein drinks have the advantage of low raw material cost, abundant sources, easy availability, and safety.

In this study, we examined the potential of using SPI and 7S (proteins extracted from soybeans) as raw materials for acid-soluble beverages. Specifically, we compared the taste of SPI and 7S prepared using ultrafiltration-diafiltration and these were prepared using double enzyme treatment. We evaluated the mouthfeel of these proteins in different beverages, including 'Gatorade' (a sports beverage), freshly squeezed orange juice, and 'Sprite' (a carbonated beverage) through sensory evaluation. We also used electronic tongue technology to observe the aggregation state of the proteins and collected human oral saliva to explore any oral rheology-related issues. The results showed that SPI and 7S produced through ultrafiltration-diafiltration exhibited improved techno-functional qualities and pleasant flavor. Therefore, these proteins can be utilized as hypoallergenic components in various acid-soluble beverages.

MATERIALS AND METHODS

Reagents

Shandong Yuwang Industrial Co., Ltd. (Yucheng, China) provided the defatted soybean meals. The protein content (47.5 ±2.1%) of defatted soybean meals was determined using the Dumas method ($N \times 6.25$, wet basis) in a nitrogen/protein analyzer (Rapid N Cube, Elementar Analysen System GmbH, Hanau, Germany), as previously reported (Yang et al., 2016). Soybean 7S meals were obtained from soybean seed varieties without Soybean 11S in the National Soybean Improvement Center Seed Bank (S11SD-12, Beijing, China, Fig. 1). The proteins of defatted soybean and soybean 7S meals were isolated, as described by Wang et al. (2020a). Sigma Chemical Co. (St. Louis, Mo., USA) provided the phytase and protease. All of the substances used in this experiment were of analytical grade.

Preparation of SPI and 7S by the ultrafiltration-diafiltration method

In this experiment, two acid-soluble soy proteins were made from low-temperature defatted soybean and enriched 7S defatted soybean meal. The extract was mixed with two soybean meals in a 1:10 (w/v) ratio with a 2 mol/L CaCl2 solution for 30 min. The solutions were stirred by a High-Speed Refrigerated

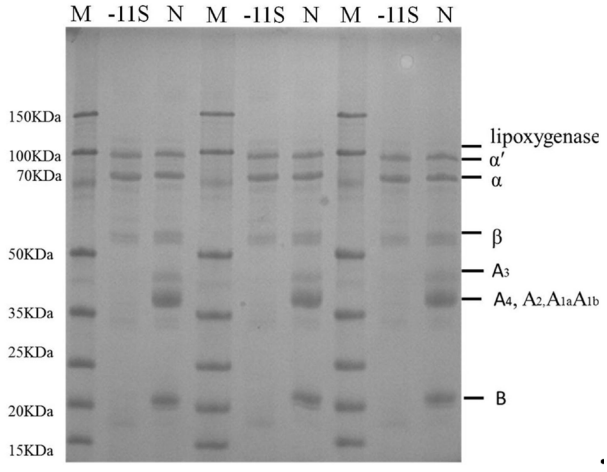


Fig. 1. SDS-PAGE pattern of soybean seed storage protein and 7S globulin. Lane 1, 4, 7: Marker protein; lane 2, 5, 8: 7S protein and lane 3, 6, 9: SPI.

Centrifuge (5,000 × g, Himac CR 22 G, HITACHI, Japan) at 25°C for 15 min. The precipitate was removed, and the supernatant was collected. 2 mol/L NaHSO3 was added to the supernatant, which stood for 15 min. The pH of the supernatant solution was adjusted to 5.0 with 1 mol/L HCl. The phytase was then added to the solutions and enzymatically treated at 50°C for 60 min. After enzymatic digestion, distilled water was added to the resolution, and the pH was adjusted to 5.0. The answers were concentrated by diafiltration-ultrafiltration in a water bath (50°C), and the distilled water was added to the concentrated solutions to restore the original volume after 10 concentrations. The process was repeated 4 times, and the distilled water was not added to the focused solutions after the last ultrafiltration. After the concentrated protein concentrate was directly pasteurized and spray-dried, the SPI and 7S were obtained and named DUD-SPI and DUD-7S.

Preparation of SPI and 7S by the double enzymatic treatment

The SPI and 7S were prepared from a low-temperature defatted soybean meal and a 7S fat-free soybean meal by adding phytase and protease, as previously described (Bae et al., 2013). The soybean meal was mixed with water at a ratio of 1:15 (w/v). The pH value of the solution was adjusted to 8.0 with 2 mol/L NaOH, stirred at 25°C for 2 h, and centrifuged at 9,000 × g by a High-Speed Refrigerated Centrifuge (Himac CR 22 G) at 25°C for 20 min and 5,000 × g at 4°C for 30 min. The pH value of the supernatant was adjusted to 4.5 with 2 mol/L HCl. After the supernatant was left at rest at 30°C and then centrifuged again at 5,000 × g at 4°C for 30 min, the pH value was adjusted to 7.0. The precipitation was re-dissolved in water, then phytase was added (pH = 4.5, 37°C, 30 min), and then protease was added for enzymatic hydrolysis (pH = 4.5, 40°C, 30 min). Finally, phytase was added, and the SPI and 7S were obtained after spray drying, named PP-SPI and PP-7S.

Preparation of complex flavor beverage

This study used three types of beverages to make protein-liquid compounds: the sports beverage 'Gatorade', a juice beverage of freshly squeezed orange juice, and carbonated beverage 'Sprite'. PP-SPI, PP-7S, DUD-SPI, and DUD-7S were placed in 12 beakers (100 mL, each sample for three cups). The 'Gatorade' was added to the beakers and stirred (called G-PP-SPI, G-PP-7S, G-DUD-SPI, and G-DUD-7S), as was the freshly squeezed orange juice (called O-PP-SPI, O-PP-7S, O-DUD-SPI, and O-DUD-7S) and the 'Sprite' (called S-PP-SPI, S-PP-7S, S-DUD-SPI, and S-DUD-7S). The SPI power was dissolved into the protein beverage at a concentration of 1% (w/v), the pH was adjusted to 3.0, then pasteurized at 90°C for 20min.

Collection of human saliva

The saliva of 10 healthy volunteers (4 men and 6 women, aged 30–35) was randomly selected. From 8:30 to 10:30 in the morning, the volunteers rinsed their mouths with water five times and then collected their saliva. Volunteers were asked to sit still, lower their heads, slightly open their mouths, and connect a graduated measuring cylinder to their lower lip, allowing saliva to flow into it naturally. All the saliva was collected within a certain period of time (e.g., 1 minute). The collected saliva was degassed and then centrifuged at 10,000 × g by a High-Speed Refrigerated Centrifuge (Himac CR 22 G) for 2 min to remove impurities. It was then placed in a refrigerator at –20°C for preservation.

Clarity analysis

The clarity of the protein can be characterized by the transparency of the appearance of the protein solution,

that is, the transmittance, which is characterized by the degree of blocking of the suspended matter in the solvent by the light. UV/VIS spectrophotometers were used to measure light transmission at specific wavelengths; clarity increases if the light transmission is high. In the experiment, the protein powder was made into a 1% (w/v) solution with distilled water and stirred until completely dissolved. A UV-VIS spectrophotometer (UV2300, Techcomp Limit., Shanghai, China) with a 10 mm pathlength quartz cuvette was used to determine its transmittance value at 600 nm.

Sensory evaluation

Sensory evaluation of the composite functional beverages was conducted by an evaluation team (consisting of 20 persons). Different blended drinks available were evaluated, and an unknown order was presented to the assessors. Gatorade, fresh orange juice, and Sprite were added into the salt extraction ultrafiltration protein and acid heat protein, respectively, and the protein added amount was 1% to prepare a composite functional beverage with a pH value of about 3.0. A sensory assessment standard table (Table 1) was used, with astringency, bitterness, and sourness being rated on a scale from 1 to 10. Higher scores indicated a stronger taste perception. The assessors objectively evaluated and graded the beverages based on the sensory evaluation standard table (Table 1).

Recognition of different functional beverages by electronic tongue

To detect the various available drinks, the study utilized an electronic tongue detection device called Tree II (Alpha-MOS, Toulouse, France), which was equipped with a food-specific sensor. For each detection, 80mL of the sample was placed in a 100mL beaker. After each test, the sensor was cleaned, and a cup of distilled water was placed between the samples during the interval.

During the experiment, the sampling time for each element was set to 120 seconds. Data were collected every second, and each piece was tested six times. To obtain stable data, the average value of the estimated value during the last 50 seconds was taken as the output value for the stable data area. The detection system consisted of seven sensors: ZZ, JE, CA, BB, DA, BA, AB, and an Ag/AgCl reference electrode. Statistical analyses, including principal component analysis and discriminant factor analysis, were performed on the original data collected by the electronic tongue.

Optical microscopy

1.0 mL of the beverage sample was quickly mixed with 1mL of saliva, and the appropriate mixture was collected to prepare the specimen. The glass specimen was placed on the objective stage of an optical microscope (BX51, Olympus, Japan) and observed with low magnification. Then the aggregate shape was maintained with a 400× magnification lens and photographed.

Viscosity determination

The viscosity of the beverage saliva mixture was determined using an arheometer (Haake RS600, Thermo, Germany). A total of 1 mL of the sample solution and 1mL of saliva were mixed together. Then, an appropriate amount of the mixture was placed between two parallel plates, with the gap between them set at 1mm. The measurement was carried out at a constant temperature of 25°C, with a continuous shear rate of 10 s-1. Any excess mixture was removed using a paper towel.

Table 1. Sensory evaluation criteria

Sensory index	0 points	1–3 points	4–7 points	8–10 points
Sour	tasteless	slightly sour, can accept	similar acidity, as fruit flavor	the sour taste of vinegar
Astringency	tasteless	slightly astringency, can accept	the tongue can feel obvious rough	it adhered to the tissue of the tongue, unacceptable
Bitterness	tasteless	slightly bitterness, can accept	there's nothing wrong with a little bitterness taste. it can be removed by drinking water	obvious bitterness, unacceptable

Statistical analysis

Unless otherwise stated, all tests were performed in duplicate or triplicate. The data were provided as a mean and standard deviation. The data were subjected to an analysis of variance (ANOVA) using the SPSS 13.0 software. The means were compared using a least significant difference (LSD) test with a 95% confidence interval. $P < 0.05$ was used to determine statistically significant differences.

RESULTS AND DISCUSSION

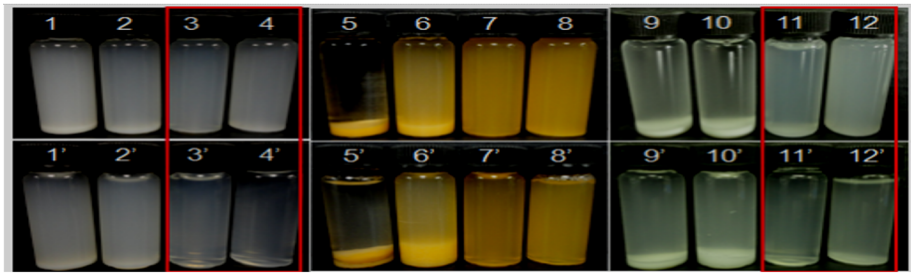
Clarification analysis of complex flavor beverage

The clarity of 12 complex flavor beverages was detected by comparing the apparent differences of different proteins in these beverages. The clarity of G-DUD-SPI and S-DUD-SPI beverages was relatively high before sterilization. After sterilization, the clarity of G-DUD-SPI, G-DUD-7S, S-DUD-SPI, and S-DUD-7S was relatively high (Table 2). Compared with "Gatorade" and "Sprite", the SPI prepared by ultrafiltration and diafiltration or double-enzymatic method was prone to precipitation when freshly squeezed orange juice was used, which may be related to the fruit juice beverage system (Cullen et al., 2004; He et al., 2015). The G-PP-SPI and G-PP-7S exhibited apparent insoluble precipitate in the four kinds of the beverage of 'Gatorade'. However, the G-DUD-SPI had higher clarity, which reached 52.36% after pasteurization. The clarity of G-DUD-7S increased from 3.80% to 59.84% before pasteurization, indicating that pasteurization improved the product clarity to some extent (Fig. 2).

Table 2. Clarify degrees of different functional beverages, %*

The samples	Before disinfection	Disinfection
G-PP-SPI	0.25 ±0.03[a]	0.14 ±0.03[a]
G-PP-7S	2.19 ±0.10[a]	1.26 ±0.23[a]
G-DUD-SPI	19.72 ±1.19[b]	52.36 ±2.12[c]
G-DUD-7S	3.80 ±0.98[a]	59.84 ±2.11[c]
O-PP-SPI	0.26 ±0.06[a]	0.20 ±0.01[a]
O-PP-7S	0.19 ±0.01[a]	0.13 ±0.05[a]
O-DUD-SPI	11.43 ±1.76[b]	20.70 ±2.33[c]
O-DUD-7S	2.57 ±1.02[b]	20.51 ±1.79[b]
S-PP-SPI	1.35 ±0.26[a]	1.26 ±0.05[a]
S-PP-7S	2.97 ±0.93[a]	2.84 ±0.03[a]
S-DUD-SPI	28.91 ±1.98[b]	59.98 ±3.12[c]
S-DUD-7S	5.13 ±1.32[b]	33.96 ±2.71[b]

*In the same table column, the means with other letters (a–c) differ significantly ($p < 0.05$). PP-SPI and PP-7S were prepared using enzymatic treatment; DUD-SPI and DUD-7S were prepared using the diafiltration-ultrafiltration method; G-PP-SPI, G-PP-7S, G-DUD-SPI, and G-DUD-7S indicate the PP-SPI, PP-7S, DUD-SPI, and DUD-7S were added to the 'Gatorade'; O-PP-SPI, O-PP-7S, O-DUD-SPI, and O-DUD-7S indicate the PP-SPI, PP-7S, DUD-SPI, and DUD-7S were added to the freshly squeezed orange juice; S-PP-SPI, S-PP-7S, S-DUD-SPI, and S-DUD-7S indicate the PP-SPI, PP-7S, DUD-SPI, and DUD-7S were added to the 'Sprite'.



Note: No.1–12 were G-PP-SPI, G-PP-7S, G-DUD-SPI, G-DUD-7S, O-PP-SPI, O-PP-7S, O-DUD-SPI, O-DUD-7S, S-PP-SPI, S-PP-7S, S-DUD-SPI, and S-DUD-7S; No. 1'~12' were the pasteurized samples of them.

Fig. 2. The clarity of 12 complexed functional beverages

Comparing these four products with carbonated beverage Sprite as the solvent, we observed that the clarification of S-DUD-SPI had a clear advantage over several other beverages, indicating that the application of the SPI prepared by ultrafiltration-infiltration was limited in fruit juice beverages. Nonetheless, there were still some improvements in the SPI by the double-enzyme method. In sports and carbonated drinks, the SPI was prepared by ultrafiltration-infiltration and maintained high clarity, which was better than the SPI prepared by the double-enzyme method.

Sensory evaluation

The sensory evaluation of 12 complex flavor beverages was detected (Abdo et al., 2020). Table 3 displays the results. Among the eight beverages tested, excluding those with freshly squeezed orange juice as a solvent, bitterness and sourness were insignificant. The reasons for astringency varied significantly among the different drinks. The SPI and 7S beverages prepared using the double-enzyme method exhibited a stronger astringency, resulting in a contraction of the tongue tip and a tingling sensation at the tongue base. The astringency scores for SPI and 7S beverages prepared through ultrafiltration-infiltration ranged from 3 to 7. The astringency of G-DUD-SPI, S-DUD-SPI, and S-DUD-7S was deemed slightly rough yet acceptable, producing an overall acceptable sensation within the human body. Thus, it can be inferred that SPI and 7S, when prepared through ultrafiltration-infiltration, possess favorable taste characteristics.

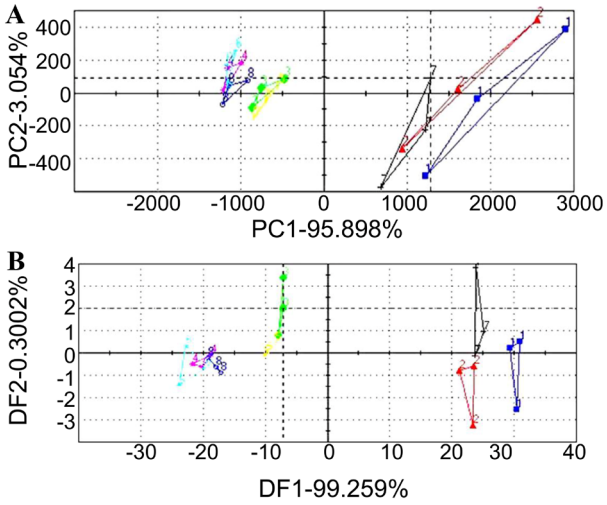
Table 3. Sensory evaluation of different beverages

The samples	Sour	Astringency	Bitterness	Total score
G-PP-SPI	2	8	0	10
G-PP-7S	1	7	0	8
G-DUD-SPI	1.25	3.75	0	5
G-DUD-7S	0.75	4.5	0	5.25
O-PP-SPI	2	9	2	13
O-PP-7S	1.5	9	2.5	13
O-DUD-SPI	5	4	3	12
O-DUD-7S	4	7.5	1	12.5
S-PP-SPI	1	8	0	9
S-PP-7S	1	6	0	7
S-DUD-SPI	1	2	0	3
S-DUD-7S	0.75	3.25	0.25	4.25

Note: PP-SPI and PP-7S, were prepared by enzymatic treatment; DUD-SPI and DUD-7S were prepared by diafiltration-ultrafiltration method; G-PP-SPI, G-PP-7S, G-DUD-SPI, and G-DUD-7S indicate the PP-SPI, PP-7S, DUD-SPI, and DUD-7S were added to the 'Gatorade'; O-PP-SPI, O-PP-7S, O-DUD-SPI, and O-DUD-7S indicate the PP-SPI, PP-7S, DUD-SPI, and DUD-7S were added to the freshly squeezed orange juice; S-PP-SPI, S-PP-7S, S-DUD-SPI, and S-DUD-7S indicate the PP-SPI, PP-7S, DUD-SPI, and DUD-7S were added to the 'Sprite'.

Electronic tongue recognition analysis

In this section, the electronic tongue detection technology and corresponding analysis mode were used to identify and study six kinds of complex functional drinks with better clarity and higher sensory scores, and the detected taste signal data were used to try to distinguish the differences between tastes. Six complex flavor beverages of G-PP-SPI, G-PP-7S, G-DUD-SPI, G-DUD-7S, S-DUD-SPI, and S-DUD-7S were used to detect the recognition. With the help of electronic tongue detection technology, S-DUD-SPI and S-DUD-7S were found to have higher clarity and sensory scores through corresponding analysis modes. The detected taste signal data is used to distinguish the difference between the original taste, and the Gatorade (or Sprite) complexed functional beverages can be used to provide more objective data support for human sensory analysis (Clyne et al., 2000; Mueller et al., 2005; Riul et al., 2002). The most common and effective principal component analysis (PCA) pattern can be used in analyzing taste signals with analysis software in the instrumental. The number of sensors was 7, and 7 sufficient samplings were performed in the 8 samples, which can better ensure the model's validity. It can be seen from Fig. 3A that the contribution rates of PC1 and PC2 in the principal components are 95.898% and 3.054%, respectively. The cumulative contribution of PC1 and PC2 is 98.952%, indicating that PCA retains most of the information in the original data and can distinguish different compound



Note: No.1–8 were G-DUD-SPI, G-DUD-7S, S-DUD-SPI, S-DUD-7S, G-PP-SPI, G-PP-7S, Gatorade, and Sprite.

Fig. 3. Analysis of (A) the principal component analysis (PCA) and (B) discriminant factor analysis (DFA) by an electronic tongue

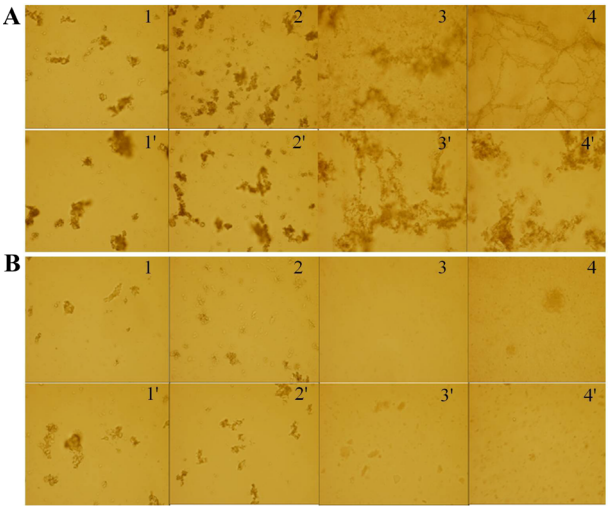
samples better. Fig. 3A shows that 8 samples (7 points of each sample after removing the 4 significant difference points) formed 8 bands on the two-dimensional spectrum, and the degree of mutual interference among the regions was small. No. 1, 2, 5, 6, and 7 were 'Gatorade' datasets, of which No. 5 and 6 were significantly different from blank No. 7, and No. 2 and 7 were closer to each other, and there is a certain degree of data crossover. No. 3, 4, and 8 were 'Sprite' datasets, of which No. 6 and 8 were relatively close, and there is a particular data intersection.

Discriminant factor analysis (DFA) is a mathematical-statistical method used to build models, identify unknown samples, and classify unknown samples (Blekherman et al., 2011; Nagy et al., 2005). A data identification model is established through mathematical transformation to maximize the distance between different sample data with differences and minimize the distance between similar sample data (Smith et al., 2006; Wang et al., 2018). In the current study, we collected signals in 8 complex flavor beverages, using DFA analysis to obtain data as shown in Fig. 3B. The contribution rates of DF1 and DF2 reached 99.259% and 0.3002%, respectively, and the cumulative contribution rate was 99.559%. Fig. 3A illustrates that datasets No. 1, 2, 5, 6, and 7 were associated with 'Gatorade', and they did not overlap with each other. On the other hand, datasets No. 3, 4, and 8 were related to 'Sprite' and had some intersections. These results indicate that the taste of the G-DUD-SPI and G-DUD-7S samples were more similar to 'Gatorade', and the addition of these proteins did not negatively impact the taste of 'Gatorade'. In contrast, the taste of the S-DUD-SPI and S-DUD-7S samples resembled 'Sprite', and the addition of these proteins did not affect the taste of 'Sprite' either. The proteins SPI and 7S were obtained through ultrafiltration-infiltration, and when added to the complex functional drinks, they allowed for the quick restoration of the original flavor of the beverage.

Optical microscopy analysis

We conducted further investigations into the aggregation of the mixture following the interaction of various proteins with human oral saliva. The formation of aggregates was observed under a magnification of 400×, using an optical microscope. Fig. 4A displays the presence of insoluble particulate matter at pH 3.5 in the PP-SPI and PP-7S solutions prior to mixing with saliva. Conversely, mesh aggregates were found in the DUD-SPI and DUD-7S solutions. Upon mixing with saliva, the particle size of PP-SPI and PP-7S increased as the particles aggregated with one another (Sun et al., 2021). In contrast, numerous small particles adhered to the grids in the cases of DUD-SPI and DUD-7S. When an acidic protein solution comes into contact with neutral saliva, an immediate interaction takes place, resulting in changes to the protein's properties. As a result, the dispersed small protein particles connect with each other to form a polymer. This phenomenon aligns with the roughness and convergence of the tongue experienced by the human body, which supports the findings of the sensory analysis conducted (Marsh et al., 2016; Sarkar et al., 2019).

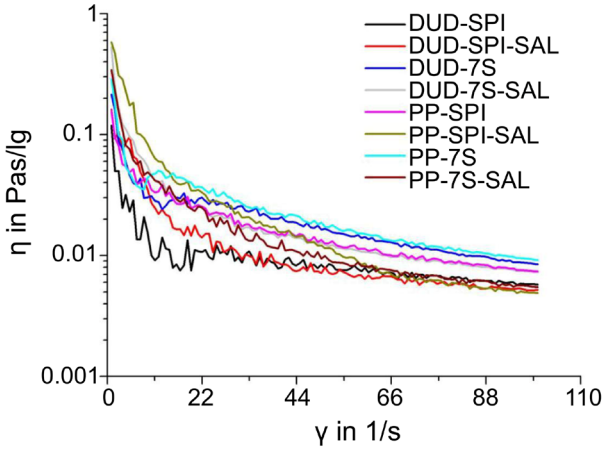
Fig. 4B illustrates the differences observed in particle aggregates, solubility, and irregularity before and after mixing with saliva at pH 7.0 for PP-SPI, PP-7S, DUD-SPI, and DUD-7S solutions. Prior to mixing, PP-SPI and PP-7S displayed fewer particle aggregates, more irregular aggregates, and higher solubility. Conversely, DUD-SPI and DUD-7S exhibited minimal changes. Previous studies hypothesized that



Note: No.1–4 were PP-SPI, PP-7S, DUD-SPI, and DUD-7S; No. 1'-4' were PP-SPI mixed with saliva, PP-7S mixed with saliva, DUD-SPI mixed with saliva, and DUD-7S mixed with saliva.

Fig. 4. Microscopic analysis of the saliva mixtures and protein samples at pH 3.5 (A) and protein samples at pH 7.0 (B)

interactions between neutral protein solutions and neutral saliva occur without significant changes in pH or protein properties, resulting in less perceived astringency and roughness on the human tongue (Carter et al., 2020; Çelebioğlu et al., 2020).



Note: DUD-SPI-SAL was DUD-SPI mixed with saliva; DUD-7S-SAL was DUD-7S mixed with saliva; PP-SPI-SAL was PP-SPI mixed with saliva; and PP-7S-SAL was PP-7S mixed with saliva.

Fig. 5. Viscosity analysis

Viscosity determination

We further investigated the mixture's viscosity after interaction with DUD-SPI, DUD-7S, PP-SPI, and PP-7S with human oral saliva (Rinaldi et al., 2012; Wang et al., 2020b). Viscosity measurements were performed using a Huck rheometer, and these protein solutions without saliva were used as a blank group. Figure 5 shows that the viscosity of PP-SPI, PP-7S, DUD-SPI, and DUD-7S mixed with saliva was slightly higher than that of the original protein solution. At the same time, the differences between PP-SPI, PP-7S, DUD-SPI, and DUD-7S were not significant. The increase in viscosity with decreasing pH was consistent with objective measurements in the model and food systems.

CONCLUSIONS

This study compared the application of DUD-SPI, DUD-7S, PP-SPI, and PP-7S in acidic beverages. The clarity, taste, microstructure, and viscosity properties of the DUD-SPI, DUD-7S, PP-SPI, and PP-7S in acidic drinks were further studied. The results showed that the clarity and taste of DUD-SPI and DUD-7S in acidic beverages were better than in PP-SPI and PP-7S. The electronic tongue identified that the effect of DUD-SPI and DUD-7S on acidic drinks was negligible. By simulating the interaction between these proteins' solution and oral saliva, it can be seen that the neutral environment of the oral cavity was the main reason for the unpleasant taste, such as astringency after eating acid. Therefore, the SPI and 7S prepared by ultrafiltration-infiltration solved this problem and could be used as hypoallergenic components in various acid-soluble beverages.

In addition to soy protein, there are many natural or denatured insoluble proteins, including fire hemp protein, pea protein, zein, and more. However, due to their poor solubility, their application field is limited. Thus, it is worth considering applying the method proposed in this research paper to process these aforementioned proteins. Moreover, further exploration is still required to investigate alternative methods for preparing acid-soluble soybean protein. The aim is to identify technologies with a simple production process, lower cost, and improved product effectiveness.

CONFLICT OF INTEREST

The authors have declared no conflicts of interest.

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