LWT - Food Science and Technology 250 (2026) 119479

Contents lists available at ScienceDirect

LWT

journal homepage: www.elsevier.com/locate/lwt

Improved textures and quality characteristics of plant-based sausages formulated with canola oil-in-water emulsions stabilized by soy protein isolate

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ARTICLE INFO

Keywords:
Plant-based meat products
Oil pre-emulsification
Soy protein-stabilized emulsions
Emulsion stability
Food texture

ABSTRACT

This study aims to investigate effects of canola oil (CO) emulsions formed by soy protein isolate (SPI) on textural properties and quality characteristics of vegetarian sausages making texturized fibril soy proteins (TFSP) as the base. Results showed that at SPI contents = 12–14 wt% and CO contents = 20–40 wt%, fine stable SPI-CO emulsions (d_{43} = 2.85–6.94 μm) could be formed, and showed an increased binding ability by increasing component contents. Compared to the direct addition of CO, the incorporation of pre-formed SPI-CO emulsions exhibited greater improvements on TPA (texture profile analysis) attributes, shear test results, and water and fat binding capacities of TFSP-based sausages, with the one formed at SPI content = 14% and CO content = 40 wt% showing the best performance. Confocal laser scanning microscopy and dynamic rheological measurements upon temperature sweeping revealed that fine stable SPI-CO emulsion droplets (<10 μm) showed a strong affinity for TFSP due to the unfolding of protein conformation at droplet interface, and behaved as active filler participating in the network formation to reinforce TFSP-based sausage matrix. This study demonstrates that pre-emulsification of oils with SPI could be an effective way to improve textures and quality characteristics of plant-based meat products.

1. Introduction

In recent years, plant-based meat products have gained considerable attention since they could be designed to approximate the texture, flavour, and appearance of real meat products, and has a broad potential to change the traditional pattern of animal products consumption (Baune et al., 2022; Kyriakopoulou et al., 2021). Plant-based sausages are one of the most representative and popular plant-based meat products, the recipe of which normally involves meat analogs, oil, binders, flavourings and water (Marianski & Marianski, 2015; Sha & Xiong, 2020). Texturized fibril soy protein (TFSP), a newly developed texturized vegetable protein (TVP), presents a meaty and chewy texture similar to animal meat and is rich in high-quality plant proteins, which makes it become popular to be used as meat analogue (Chen et al., 2021; Luo et al., 2024). However, attempts to manufacture TFSP-based meat products have resulted in undesirable textures and quality attributes (Chen et al., 2021; Luo et al., 2024). This may be due to the fact that although soya based texturized proteins resemble the texture of meat, they are less effective than meat proteins in providing binding, emulsifying and gel-forming characteristics that are important in the processing of meat products (Bakhsh et al., 2021; Lin & Barbut, 2025; Samard & Ryu, 2019).

Traditional sausages are primarily composed of lean meat, adipose tissue, water and salt, which are all finely chopped together to obtain a homogeneous mass. Through this operation, fat is chopped into small globules, which are covered by a membrane coating made largely of salt-soluble myofibrillar proteins (Gordon & Barbut, 1992). Structurally, emulsified lipids are dispersed in the network of protein gel matrix (batter) and act as fillers or copolymers, thereby playing an important role in the rheological and structural properties as well as providing a unique taste profile (Jiang & Xiong, 2015). Replacing animal fat with vegetable oil delivers good results, provides the products with a better fatty acid profile and lower cholesterol level compared to the traditional ones. However, unlike animal fat, vegetable oils rich in unsaturated fatty acid are liquid when heated and remain liquid when cold. Previous studies have found that the direct incorporation of oils into sausages

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https://doi.org/10.1016/j.lwt.2026.119479
Received 23 December 2025; Received in revised form 19 March 2026; Accepted 8 May 2026
Available online 13 May 2026

often results in poor binding and texture may crumble (Bolger et al., 2018; Majzoobi et al., 2017). This is because the high fluidity of vegetable oils entails strong shear force or high pressure homogenization to produce protein-coated stable oil droplets. However, the extremely high viscosity of sausage batters technologically prohibits the creation of such stable emulsions with a cohesive interfacial membrane (Jiang & Xiong, 2015). Therefore, the idea of manufacturing plant-based sausages by reformulating traditional food is technologically challenging. As the base ingredients of plant-based sausages are different from those of meat sausages, new procedure must be introduced to bypass the established ways of making sausages (Baune et al., 2022; Kyriakopoulou et al., 2021).

Pre-emulsification of oils has been used in sausage manufacturing when incorporating fat/oil that are difficult to stabilize (Jiménez-Colmenero, 2007; Jiménez-Colmenero et al., 2010). The commonly applied procedure is one proposed by Jiménez-Colmenero (2007), and in this case, a water phase containing a plant-derived emulsifier is homogenized with an oil phase, which leads to the formation of emulsifier-coated oil droplets. Soy protein isolate (SPI) is commonly used as emulsifier in pre-emulsification process because this plant-derived protein allows to emulsify different types of oils, and their ability to produce gel contributes to the increased firmness of the sausage matrix (Bolger et al., 2018; Kothuri et al., 2024; Luo et al., 2024). Canola oil (CO) is rich in unsaturated fatty acids and linolenic acid that provide health benefits, and has a reported positive effect on improving textures of plant-based meat products due to its relatively high viscosity among conventional vegetable oils (Kothuri et al., 2024; Youssef & Barbut, 2011). However, the effects of pre-emulsified canola oil with SPI on the textural properties of TVP-based meat products seem ambiguous. As Kothuri et al. (2024) reported, the incorporation of pre-emulsified canola oil with SPI caused an increase in TPA hardness and cohesiveness of TVP-based batters, while Luo et al. (2024) found that such a treatment led to less retained texture profile of TVP-based batters. It is noteworthy that emulsion formulations (e.g., SPI contents and CO contents) used in these studies were different, which could result in emulsions presenting different technological characteristics (droplet size, viscosity, stability, etc.). However, the technological characteristics of emulsions played a critical role in affecting the textures and quality characteristics of emulsified meat products (Keum et al., 2024; Wu et al., 2011; Youssef & Barbut, 2011). Therefore, a systematic study is needed to identify the influences of changes in technological characteristics of pre-formed emulsions on the textures and quality characteristics of TFSP-based meat products. However, to our knowledge, limited research on this topic has been reported so far in literature.

In this study, a series of canola oil emulsions emulsified with SPI (SPI-CO emulsions) were prepared by varying SPI contents (10–16 wt%) and canola oil contents (20–50 wt%) during emulsification, and were then incorporated into TFSP-based sausages. Emulsion droplet sizes, apparent viscosities, and microstructures of SPI-CO emulsions were determined and their effects on textures and water and fat binding (WFB) capacities of TFSP-based sausages were investigated. Heat-induced gelation properties and microstructures of TFSP-based sausages were studied in order to explore the underpinning mechanism of their improved textures and quality characteristics caused by the incorporation of pre-formed SPI-CO emulsions.

2. Materials and methods

2.1. Materials

Texturized fibril soy protein (TFSP) was obtained from Baichuan Food Co. Ltd. (Wenzhou, China); according to the information provided by the manufacturer, this TFSP contains (g/100g of TFSP product) 66.2 protein, 20.5 carbohydrate, 1.9 fat; the moisture content is 5.8%, determined according to AOCS Official Method Ba 2a-38. Soy protein isolate (SPI; SUPRO® EX37-HG-IP) was obtained from Solae Dupont (Geneva, Switzerland); according to the information provided by the manufacturer, this SPI contains >90% protein (w/w, dry basis), <6.0% moisture, <1.0% fat, <5.0% ash. Canola oil was purchased from a local supermarket (Guangzhou, Guangdong province). Nile Blue and Nile Red were purchased from Sigma Chemicals (St. Louis, MO, USA). All other chemicals used were of analytical grade and commercially available. Water purified with a Milli-Q filtration unit (Millipore, Bedford, UK) was used for the preparation of solutions or sample dispersions.

2.2. Preparation and characterization of SPI-canola oil emulsion (SPI-CO emulsion)

The SPI-CO emulsions used for incorporating into TFSP-based sausage were prepared according to the method of Jiménez-Colmenero et al. (2010), with slight modifications. SPI powder was dispersed into deionized water to reach different concentrations (10.0–16.0 wt%) and was stirred using a Hobart N50 laboratory mixer (Hobart MFG. Co., Troy, USA) for 60 min to ensure complete dispersion, followed by storage overnight at 4 °C. The following day, the SPI dispersion was mixed using a high-speed homogenizer (Fluko FA25, Germany) operating at 25,000 rpm for 5 min, with the gradual addition of canola oil into the mixture. In the resultant SPI-CO emulsions, the CO content ranged from 20 to 50 wt%. Emulsion sample codes are subsequently composed of the SPI content in SPI dispersion and the CO content in SPI-CO emulsion. For example, SPI14%-CO40% refers to the SPI-CO emulsion contained 40 wt% canola oil and prepared using SPI dispersion with 14 wt% SPI.

Droplet size distributions (DSD) of emulsions were measured by static multi-angle light scattering using a Mastersizer 3000 (Malvern Instruments Ltd., Worcestershire, UK), with absorption index of 0.001. Refractive indices of canola oil and water were taken as 1.471 and 1.330, respectively. Mean droplet sizes were characterized as volume mean diameter $d_{43} = \sum n_i d_i^4 / \sum n_i d_i^3$, where n_i is the number of droplets of diameter d_i.

Apparent viscosities of emulsions were measured using a Paar Physica MCR 301 rheometer (Anton Paar GmbH, Graz, Austria) equipped with a Peltier plate temperature control unit (P-PTD 200) and a parallel and plate geometry (PP 50-1/TG; diam. 50 mm). Viscosity measurements were conducted in a rotation mode with an operating gap of 1.0 mm. For each measurement, sample was first pre-sheared at a constant shear rate of 1 s^{-1} for 30 s and then allowed to rest for 1 min on the rheometer stage. Apparent viscosity was measured at 25 °C from 0.01 to 100 s^{-1}.

Microstructures of emulsions were visualized using a LSM 710 confocal laser scanning microscope (Carl Zeiss Microscopy GmbH, Jena, Germany) in fluorescence mode. An aliquot (20 μL) of Nile Red dye solution (0.2 mg/L dye in 1,2-propanediol) was added to 5 mL of emulsion, with magnetically stirring for 5 min. The stained emulsion samples were scanned using a 40 × objective lens with numerical aperture 1.20, and was excited with 488 nm Argon laser line. Fluorescence intensity data were collected in a channel corresponding to 653–750 nm. As Nile Red stained the oil phase, individual large oil droplets and regions rich in emulsion droplets appear as greenish colour, whilst the aqueous (water/protein) phase appears dark in microimages. At least three images were obtained for each treatment, and were analyzed by Zen 3.3 software (Blue edition).

2.3. Preparation of TFSP-based sausages

TFSP-based sausages were prepared according to a basic formulation of emulsion-type vegetarian sausage described by Marianski and Marianski (2015). As shown in Table 1, the total amount of each batch of sausage batter was 1000 g, and each batch was prepared with the constant amount of rehydrated TFSP (540 g) and pre-formed SPI-CO emulsions (300 g). The CO content in different SPI-CO emulsions varied from 20 to 40 wt%, which was added in sufficient amount to give final

Table 1
The experimental formulations of TFSP-based vegetarian sausages.

Ingredients	Weight in batch (g)	Mass percentage in the finished sausage (wt.%)
Rehydrated TFSP (constant)	540.0	54
Pre-formed SPI-CO emulsions [a]	300.0	30
SPI (variable) [b]	18.0–38.4	—
Canola oil (variable)	60.0–120.0	6.0–12.0
Water for pre-emulsion (variable)	151.2–216.0	—
Others	160.0	16
SPI (variable) [b]	11.6–32.0	5.0
NaCl (constant)	10.0	1.0
Added water (variable)	118.0–138.4	—
The total amount of each batch (constant)	1000	

[a] each batch of TFSP-based sausages was prepared with the same amount of 300 g pre-formed SPI-CO emulsion.
[b] the total SPI content in all of different TFSP-based sausages was 5.0 wt%, including SPI present in the pre-formed SPI-CO emulsion and any additional SPI added.

canola oil levels of 6.0–12.0 wt% in the sausage products. Additional SPI was added, which gave final SPI content of 5.0 wt% in the sausage products. To avoid interference, no binding agents or colorants were added to the TFSP-based sausages. Consequently, the textures and quality characteristics of TFSP-based sausage samples were primarily dependent on TFSP, SPI-CO emulsion, and the interaction between them.

TFSP was rehydrated as recommended by the manufacturer. Briefly, 180 g of TFSP chunks were soaked in deionized water for 2 h. The extra water was drained off using a manual screw dehydrator to ensure that the hydrated TFSP samples were 540 ± 0.1 g in weight (1 part of TFSP adsorbed 2 parts of water). The hydrated TFSP chunks were comminuted using a bowl cutter (Blixer® 4 V.V., Robot Coupe, France) at 3500 rpm for 5 min, and was then mixed with SPI-CO emulsion and other ingredients for another 5 min. The resulting batter was stuffed into water impermeable plastic casings with a diameter of 30 mm. The sausage pieces were 10 ± 0.1 cm in length and 70 ± 0.2 g in weight, and the ends of each piece were sealed tightly. The sausage pieces were put into a steamer and cooked at 100 °C for 30 min. After the cooking treatment, the sausage samples were cooled to room temperature and stored at 4 °C in a refrigerator for at least 20 h. Sausage sample codes are subsequently composed of the name of sausage base and the code of pre-formed SPI-CO emulsions. For example, TFSP-SPI14%-CO40% refers to the TFSP-based sausage prepared with SPI14%-CO40% emulsion. An oil-free TFSP-based sausage prepared according to the formulation and procedures described above was used as control group 1. Control group 2 was prepared according to conventional processing procedures of emulsion-type sausages (Marianski & Marianski, 2015). Briefly, SPI dispersions and hydrated TFSP were prepared as described above, and were then mixed together and finely chopped using the Blixer bowl cutter at 3500 rpm for 5 min. The canola oil was then added directly to the mixture and was emulsified at 3500 rpm for another 5 min until a homogeneous mass was formed. The following procedures were the same as described above for the experimental groups. All batches were made in duplicate.

2.4. Textural analysis

The textural properties were evaluated using a texture analyzer (TA-XT2i, Stable Micro System Ltd., Surrey, UK). Casings of sausage samples were removed carefully before the textural analysis. Texture Profile Analysis (TPA) was performed on 20 mm thickness-section cut from sausage samples using a cylindrical probe (P/36R). Sections of sausage samples were axially compressed in two consecutive cycles at 50% of its original height with 5 s interval between cycles. TPA tests were performed under 3.0 mm/s pre-test speed, 1.0 mm/s test speed, 1.0 mm/s post-test speed, and trigger type-auto/force 5 g. The following parameters were calculated from the force-deformation curve: Hardness (N); Springiness (ratio); Cohesiveness (ratio); Chewiness (N) = Hardness × Cohesiveness × Springiness (Caine et al., 2003).

Shear test was performed using a Warner-Bratzler 'V' blade (A/LKB) attached to the same texture analyzer. Thirty-millimeter thickness-section was cut from sausage samples, and shear tests were performed under 3.0 mm/s pre-test speed, 1.0 mm/s test speed, 1.0 mm/s post-test speed, trigger type-auto/force 5 g, and the shear distance was 70% of the height (diameter) of sausage samples. The parameter recorded was the maximum shear force (the highest peak in the force-deformation curve) and the work of shearing (the area under the curve).

2.5. Determination of water and fat binding (WFB) capacity

The WFB capacities of TFSP-based sausages were measured according to the protocol described by Bolger et al. (2018), with minor modifications. Approximately 30 g of raw sausage batter was weighted carefully and was transferred to an 80 mL centrifuge tube with a plastic cap. After the air bubbles were removed by centrifuging (1000×g, 3 min), the tubes were place in a water bath at 95 °C for 45 min. On removal the tubes were placed on ice/water for 10 min to cool to room temperature. The tubes were then left to stand upside-down for 1 h to release the separated oil and/or water onto an evaporating dish. The total fluid loss (TFL) was measured as % of initial sample weight: TFL = $(W_F/W_T) \times 100\%$, where W_F is the mass of total fluid released (g), W_T is the initial mass of sausage batter (g). Water loss (WL) was determined as % weight loss after heating the total released fluid in an oven at 100 °C for 16 h. The WL (%) was expressed as a percentage of the initial sausage batter (g). Fat loss (FL) was calculated by subtracting the WL from TFL and WR, ignoring any minor salt component found in the dried evaporating dish.

2.6. Dynamic rheological measurements of TFSP-based sausage batters

The storage modulus G' of TFSP-based sausage batters were continuously monitored during the heat-induced gelation using a Paar Physica MCR 301 rheometer (Anton Paar GmbH) equipped with a Peltier plate temperature control unit (P-PTD 200) and a parallel and plate geometry (PP 50-1/TG; diam. 50 mm). Small-deformation rheometry was set in an oscillatory mode with an operating gap of 2.0 mm. A thin layer of silicone oil and lubricated O-ring were put around the bottom plate edge to prevent evaporation. The temperature cycle was programmed to heat the samples from 25 to 100 °C (2 °C/min), held at 100 °C for 20 min, and then cooled from 100 to 25 °C at 2 °C/min. During the temperature cycle, the oscillatory strain of 1% (within the linear visco-elastic region) was applied at 1 Hz frequency. Evolution of G' was recorded by Start Rheoplus software during the whole period of measurement.

2.7. Confocal laser scanning microscopy (CLSM) observations of TFSP-based sausages

Microstructures of sausages were visualized using a LSM 710 CLSM instrument (Carl Zeiss Microscopy GmbH) according to a procedure described by Liu and Lanier (2015), with minor modifications. The oily substance in sausage samples was stained with 0.2% mg/L Nile Red dye (dissolved in 1,2-propanediol), and proteinaceous substance was stained with 0.2% mg/L Nile Blue dye (dissolved in deionized water). Sausage samples were cut into thin slices (5 mm × 5 mm × 1 mm thick), and the Nile Red solution (20 μL) and Nile Blue solution (20 μL) were pipetted onto the cut surface of each sausage slice. The dye was allowed to absorb into the sausage slices at room temperature for 20 min. The stained sausage samples were scanned using a 40 × objective lens with numerical aperture 1.20, and were excited at 488 and 633 nm, respectively. Fluorescence intensity data for Nile Blue and Nile Red were collected in two separate channels corresponding to 503–613 and 653–750 nm, respectively. The oily substance and the proteinaceous substance in sausage samples appeared as greenish colour and reddish colour in microimages, respectively. At least three images were obtained for each treatment, and were analyzed by Zen 3.3 software (Blue edition).

2.8. Statistical analysis

Unless otherwise stated, all the tests were conducted in triplicate, and results were presented as mean ± standard errors. Significant differences between treatment means were identified by a one-way analysis of variance (ANOVA) and determined using a Tukey test ($P < 0.05$).

3. Results and discussion

3.1. Effects of SPI content and canola oil (CO) content on the technological characteristics of SPI-CO emulsions

Effects of SPI content and CO content on mean droplet sizes d_{43} of SPI-CO emulsions are shown in Fig. 1a, and CLSM images of SPI-CO emulsions with their droplet size distributions (DSD) superimposed are shown in Fig. 2. At SPI content = 10.0 wt%, the difference among d_{43} values of SPI10%-CO group was not statistically significant ($P > 0.05$) as the CO content increased from 20 to 40 wt%. Microstructural observations showed that droplets in SPI10%-CO20-40% were fine (d_{43} = ca. 2.7 μm) and homogeneous, with DSD distributing mainly in the range of 1–10 μm. However, with a further increase of CO content up to 50 wt%, SPI10%-CO50% emulsion showed a large d_{43} value of 25.1 μm, and appeared to flocculate extensively and contain a lot of big droplets. Macroscopically, oiling-off was observed in SPI10%-CO50% emulsion (see Fig. 1c). In fact, extensive droplet flocculation, coalescence and oiling-off were observed in all SPI-CO emulsions prepared at CO content = 50 wt%, no matter which SPI content (10–16 wt%) was used, indicating that a canola oil content of 50 wt% was too high to be emulsified effectively by SPI. As the CO content increased from 20 to 40 wt%, the d_{43} values of SPI12%-CO group increased from 2.85 to 4.45 μm, and those of SPI14%-CO group increased from 4.36 to 6.94 μm. Microstructural observations showed that although the DSD of SPI12%-CO and SPI14%-CO both showed an increase in big droplets (>10 μm) as the CO content increased from 20 to 40 wt%, the droplets in SPI12-14%-CO20-40% were still distributed mainly in the range of 1–10 μm with no obvious sign of droplet flocculation. Considering that fine stable SPI-CO emulsions could be formed at SPI content = 10 wt% and CO content ≤40 wt%, the increase in droplet sizes of SPI12%-CO20-40% and SPI14%-CO20-40% was probably not caused by insufficient SPI content, but rather by the increased viscosity caused by increasing SPI content and CO content. It has been reported that the increase in viscosity of component phases posed a negative effect on the rates of droplet disruption and emulsifier adsorption during emulsion formation, leading to an increase in emulsion droplet size (Gu et al., 2009). For

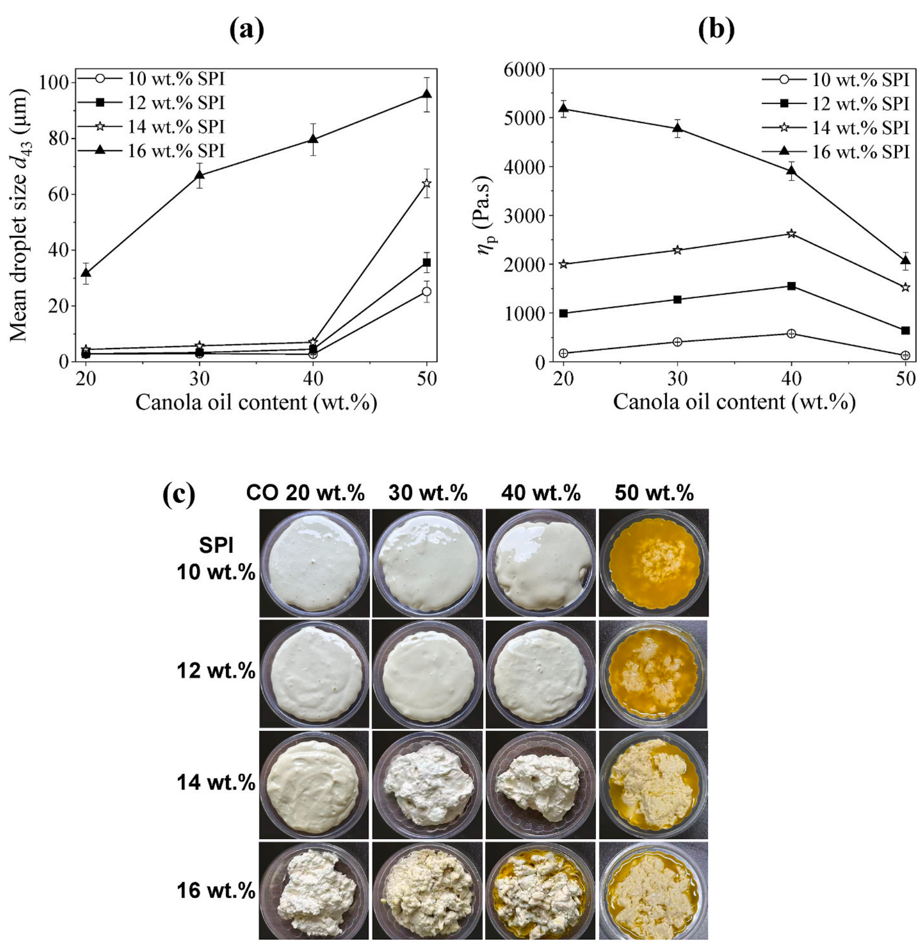

Fig. 1. Influences of SPI content (10–16 wt%) and canola oil content (20–50 wt%) on the mean droplet size d_{43} (a), viscosity η_p (b) and visual appearance (c) of SPI-CO emulsions.

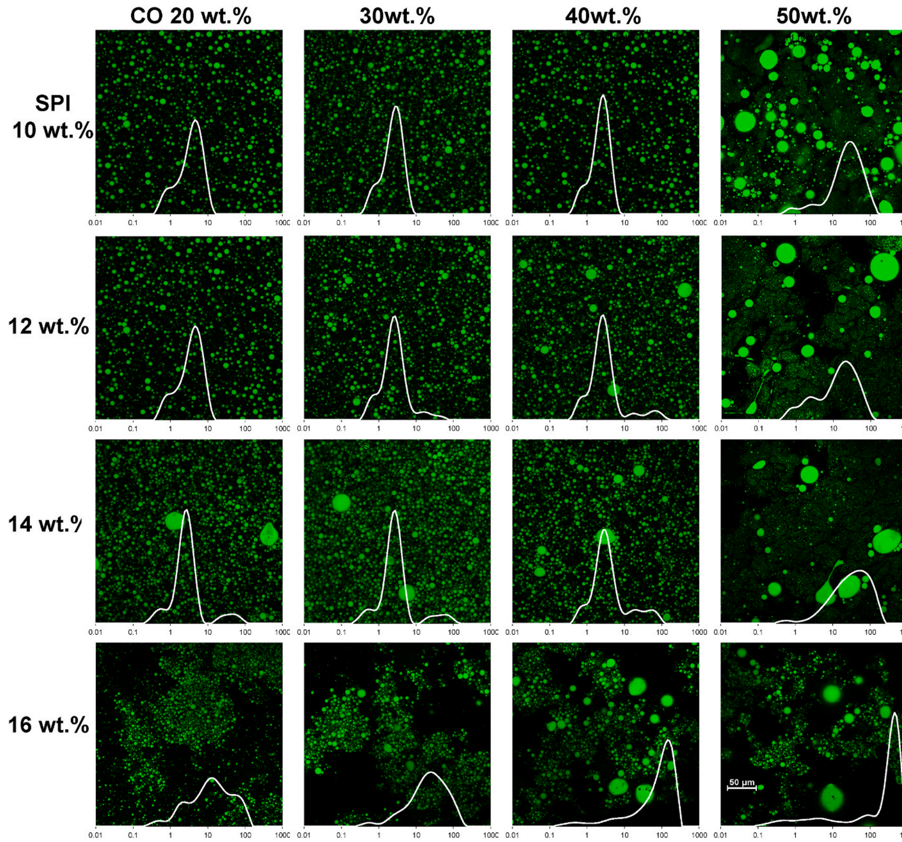

Fig. 2. Influences of SPI content (10–16 wt%) and canola oil content (20–50 wt%) on the microstructures of SPI-CO emulsions. Droplet size distributions of diluted emulsions determined by light scattering (Mastersizer) are superimposed on the CLSM microimages, with horizontal scale indicating particle size in micrometers. Bar = 50 µm.

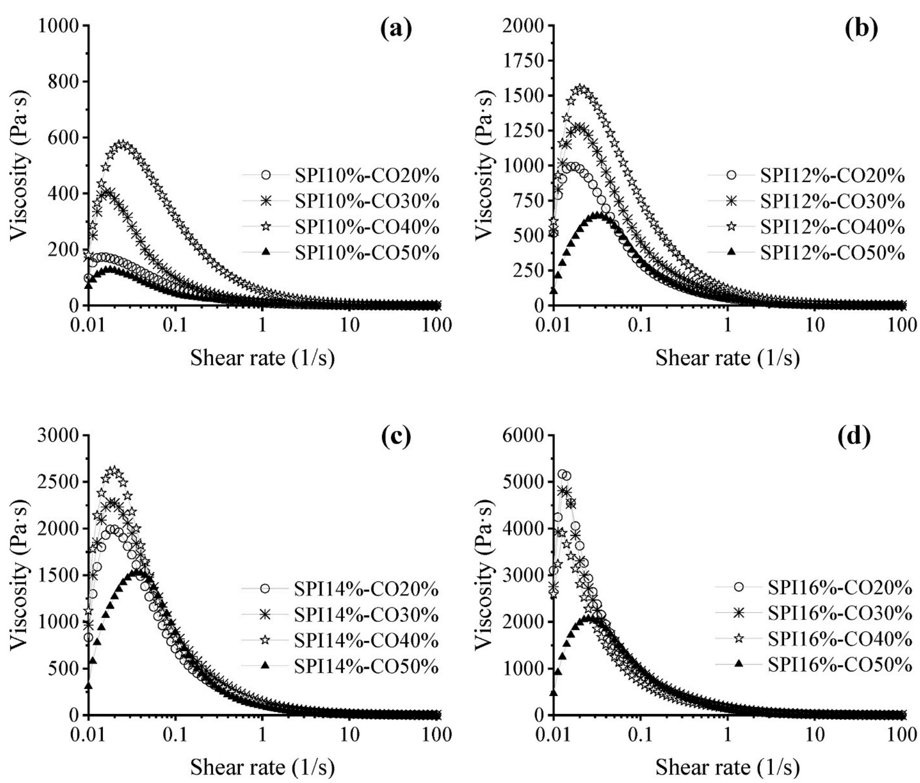

Fig. 3. Viscosity *vs.* shear rate profile of SPI-CO emulsions prepared with different contents of SPI and canola oil. (a) SPI 10%-CO20-50%; (b) SPI 12%-CO20-50%; (c) SPI 14%-CO20-50%; (d) SPI 16%-CO20-50%.

SPI16%-CO20-50%, droplet flocculation was clearly evident, and they all showed an extraordinarily high d_{43} value (>30 μm). These results indicated that stable SPI-CO emulsions could not be formed at SPI content of 16 wt%, probably because 16 wt% SPI dispersion was too viscous to emulsify canola oil effectively.

The viscosities of SPI-CO emulsions prepared with different content of SPI and canola oil against shear rate are demonstrated in Fig. 3a–d. It is seen that flow curves of all SPI-CO emulsions exhibited three distinct regions: Region 1, where at the lowest shear rates, the emulsions showed a tendency towards increased viscosity. A small shear rate-dependent plateau region was visible (Region 2) as was a decrease in viscosity at higher shear rates (Region 3). In Region 1, the increase in viscosity at low shear rates likely resulted from weak network formation via droplet flocculation and weak associative interactions (Firoozmand & Rousseau, 2016). In Region 2 (0.01–0.1 s^{-1}), SPI-CO emulsions showed distinct plateau viscosity values (η_p), which could be used as an indicator of their binding ability (Ningtyas et al., 2021). When the η_p values derived from the rheological measurements were analyzed and the mean values were compared (Fig. 1b), it was found that the more SPI content SPI-CO emulsions contained, the higher the η_p they will be presented. As shown in Fig. 1c, the morphology of SPI-CO emulsions changed from a liquid to gel as the SPI content increased from 10 to 16 wt%, and the oil content was another important factor affecting the binding ability of SPI-CO emulsions. The η_p values of SPI10-14%-CO20-50% increased as the CO content increased from 20 to 40 wt%, and then decreased markedly at 50 wt% CO content. This finding was consistent with those of Roesch and Corredig (2002), who reported that as the increase of oil content in emulsions formed by soy protein concentrate, the hydrophobic attractions between oil droplets increased because they were more closely packed with each other, which caused an increase in emulsion viscosity. The SPI14%-CO40% emulsion had a η_p value of 2620.7 Pa s and resembled a mayonnaise-like gel that could support its own weight (see Fig. 1c). The increased binding ability of SPI-CO emulsions might help to bind different ingredients together in emulsified sausages (Kyriakopoulou et al., 2021). In contrast, the η_p of SPI16%-CO group decreased gradually as the oil content increased from 20 to 50 wt%, suggesting that the use of excessive SPI and/or CO posed a negative effect on the stability of SPI-CO emulsions, and caused a marked decrease in their binding ability. Taking the droplet size, viscosity and stability of different SPI-CO emulsions into account, the emulsion formulations of SPI12%-16%-CO20-40% were chosen to make TFSP-based sausages for further investigation.

3.2. Effects of pre-formed SPI-CO emulsions on the textures of TFSP-based sausages

The textural properties are crucial for developing plant-based meat products, because a desirable texture is an essential factor in mimicking the organoleptic taste of real meat products. The TPA parameters of TFSP-based sausages formulated with different SPI-CO emulsions are shown in Table 2. Except for springiness, the hardness, cohesiveness, and chewiness of control group 1 (TFSP-based sausage prepared without canola oil) were significantly ($P < 0.05$) lower than those of other sausage samples. Previous studies have reported that plant-based meat products produced by soya based texturized proteins obtained a very soft and unacceptable texture, because they lack binding, emulsifying and gel-forming function that are important in the processing of meat products (Bakhsh et al., 2021; Lin & Barbut, 2025; Samard & Ryu, 2019). The hardness and cohesiveness of control group 2 (TFSP-based sausage prepared with direct-added canola oil) increased by 15.67% and 9.52% compared to those of control group 1. As shown in Fig. 4, control group 2 still presented a loose sausage matrix and poor sliceability. These results indicated that direct incorporation of canola oil into TFSP-based sausage during the chopping procedure only produced a limited improvement on the textural properties of sausage products.

In contrast, when canola oil was pre-emulsified by SPI and was then incorporated as SPI-CO emulsions, they will influence the TPA parameters of TFSP-based sausages to varying extents, depending on the

Table 2
Results of TPA test and shear test of TFSP based sausages formulated with different SPI-CO emulsions [a].

SPI content (wt. %)	Oil content (wt. %)	Results of TPA test				Results of shear test	
		Hardness (g)	Springiness (dimensionless)	Cohesiveness (dimensionless)	Chewiness (g)	Maximum shear force (g)	Work of shear (g. s)
12.0	20.0	768.22 (±8.50) f	0.63 (±0.01) d	0.53 (±0.01) c	256.63 (±9.59) d	494.30 (±4.77) f	5906.93 (±51.16) f
	30.0	886.93 (±9.26) e	0.64 (±0.01) cd	0.55 (±0.01) b c	312.33 (±11.28) c	518.49 (±5.16) e	6139.87 (±53.04) e
	40.0	975.16 (±7.18) c	0.66 (±0.01) c	0.57 (±0.02) b	367.08 (±17.26) b	543.51 (±5.45) c	6437.29 (±58.36) c
14.0	20.0	952.63 (±8.65) d	0.65 (±0.02) cd	0.57 (±0.01) b	353.18 (±16.54) b	529.83 (±4.25) d	6298.57 (±53.26) d
	30.0	1022.48 (±9.37) b	0.68 (±0.01) a	0.61 (±0.01) a	424.27 (±13.94) a	562.10 (±4.37) b	6580.14 (±60.14) b
	40.0	1097.20 (±9.62) a	0.69 (±0.01) a	0.62 (±0.01) a	469.62 (±20.43) a	583.02 (±4.68) a	6942.55 (±64.02) a
16.0	20.0	673.64 (±10.81) g	0.57 (±0.01) e	0.48 (±0.01) d	184.43 (±8.19) e	408.35 (±9.74) g	4944.62 (±76.40) g
	30.0	662.25 (±7.63) gh	0.56 (±0.01) ef	0.47 (±0.02) de	174.64 (±10.24) ef	403.52 (±11.58) gh	4859.17 (±59.35) gh
	40.0	638.40 (±10.58) h	0.54 (±0.02) ef	0.45 (±0.01) e	155.32 (±10.08) f	379.48 (±10.31) h	4776.25 (±65.49) hi
Control group 1 [b]		537.28 (±7.41) i	0.53 (±0.02) f	0.42 (±0.01) f	119.74 (±7.36) g	314.65 (±4.79) i	3857.25 (±64.80) j
Control group 2 [c]		621.46 (±8.07) h	0.54 (±0.01) f	0.45 (±0.01) e	151.11 (±6.63) f	368.82 (±6.41) h	4722.65 (±51.63) i

[a] In the comparison of the same type of index, results having different letters are significantly different ($P < 0.05$).
[b] Control group 1: TFSP-based sausage prepared without canola oil.
[c] Control group 2: TFSP-based sausage prepared with direct-added canola oil and the sausage formulation is the same as TFSP-SPI14%-CO40%.

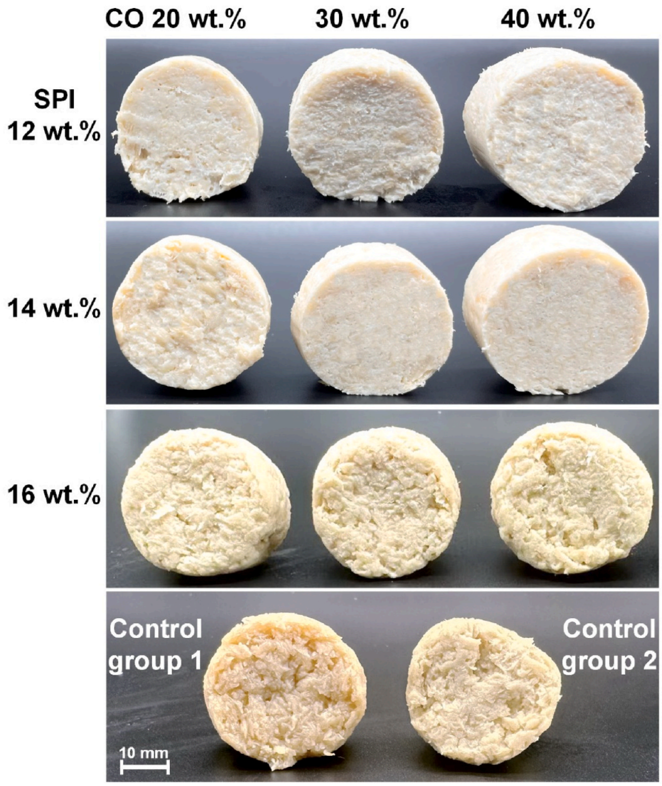

Fig. 4. Visual appearance of TFSP-based sausages formulated with different SPI-CO emulsions. Control group 1: TFSP-based sausage prepared without canola oil; Control group 2: TFSP-based sausage prepared with direct-added canola oil and the sausage formulation is the same as TFSP-SPI14%-CO40% (the oil content in sausage is 12 wt%). Bar = 10 mm.

viscosity and stability of SPI-CO emulsions. Generally, TFSP-based sausages prepared with SPI-CO emulsions presented significantly ($P < 0.05$) higher hardness, springiness, and cohesiveness than the control groups. For TFSP-SPI12-14%-CO20-40%, all tested TPA parameters showed an increased tendency by increasing SPI content and CO content in SPI-CO emulsions. The hardness, springiness, and cohesiveness of TFSP-SPI14%-CO40% increased by 76.55%, 28.07%, and 35.42% compared to those of control group 1. The increase in hardness of emulsion-type sausage indicates an increase in the strength of protein network, and the increase in cohesiveness indicates that the tightness of the combination between components in the sausage matrix was increased (Schreuders et al., 2021). The secondary parameters of chewiness behaved similarly to the parameters on which they were dependent showing an increase related to SPI content and oil content. In addition, as shown in Fig. 4, the sausage slice of TFSP-SPI14%-CO40% appears to be more compact, smoother and plumper than those of control groups. Therefore, it is clear that the textural properties of TFSP-based sausages were markedly improved when fine stable SPI-CO emulsions were introduced. These results are in accordance with those noticed by several authors who showed a rise in the TPA attributes of meat products when pre-emulsification technology was applied (Jiménez-Colmenero et al., 2010; Kang et al., 2023; Youssef & Barbut, 2011). It has been suggested that compared to the direct addition of oil, the incorporation of pre-emulsified oil enhanced the fat/oil binding capacity, and reinforced the physical stability of meat protein matrix, thereby improving the textural properties of final meat products (Kang et al., 2023; Youssef & Barbut, 2011). However, with a further increase of SPI content up to 16 wt%, although an increase in η_p was found for SPI16%-CO emulsions, a diminution in hardness, springiness, and cohesiveness was observed for TFSP-SPI16%-CO sausages. Visual observation also found that the sausage matrix of TFSP-SPI16%-CO became less compact and had some large cavities. These results may be related to the fact that SPI16%-CO emulsions were unstable, because extensive droplet flocculation and coalescence were occurred.

Shear test results are shown in Table 2 as two parameters: maximum shear force and work of shearing. Maximum shear force is defined as the force to cut the sample while work of shearing is defined as the work needed to move the blade through the sample. The lowest maximum shear force and work of shearing values were determined in control group 1, while the highest maximum shear force and work of shearing values were found in sausages produced with SPI14%-CO40%. Warner-Bratzler shear force is obtained by the maximum force required to shear samples while TPA hardness is defined as the maximum force required to compress samples (Caine et al., 2003). The trends determined in TPA hardness values were also determined for the values of maximum shear force, suggesting that there was an increase in the strength needed to break and compress TFSP-based sausages when SPI-stabilized canola oil emulsions were introduced. The different textural behaviours observed in TFSP-SPI-CO sausages show the potential of pre-formed SPI-CO emulsions in determining the textural properties of final sausage products.

3.3. *Effects of pre-formed SPI-CO emulsions on the WFB capacities of TFSP-based sausages*

Water and fat binding (WFB) capacity measures how well the juices are retained in sausage products, and were measured by determining the TFL, WL and FL (Bolger et al., 2018). As shown in Table 3, control group 1 showed the highest TFL (8.51%) and highest WL (8.51%) in all samples. Direct addition of canola oil during chopping procedure reduced the TFL and WL of TFSP-based batters (control group 2), but resulted in a high FL rate (2.16%). Previous studies have reported that soya based textured protein could only form a loose gel matrix and was not able to

Table 3
Water and fat binding (WFB) capacities of TFSP-based sausages formulated with different SPI-CO emulsions [a].

SPI content (wt%)	Oil content (wt%)	Total fluid loss (%)	Water loss (%)	Fat loss (%)
12.0	20.0	4.22 (±0.26) [d]	3.85 (±0.18) [c]	0.37 (±0.04) [d]
	30.0	4.02 (±0.17) [d]	3.61 (±0.14) [c]	0.41 (±0.06) [d]
	40.0	3.67 (±0.13) [e]	3.28 (±0.10) [de]	0.39 (±0.05) [d]
14.0	20.0	3.93 (±0.24) [de]	3.57 (±0.17) [cd]	0.36 (±0.03) [d]
	30.0	3.57 (±0.16) [ef]	3.18 (±0.15) [e]	0.39 (±0.03) [d]
	40.0	3.31 (±0.10) [f]	2.92 (±0.07) [f]	0.39 (±0.02) [d]
16.0	20.0	5.28 (±0.37) [c]	4.25 (±0.31) [b]	1.03 (±0.09) [c]
	30.0	5.14 (±0.28) [c]	4.05 (±0.25) [b]	1.09 (±0.06) [c]
	40.0	5.95 (±0.44) [bc]	4.31 (±0.36) [b]	1.64 (±0.11) [b]
Control group 1 [b]		8.51 (±0.94) [a]	8.51 (±0.94) [a]	—
Control group 2 [c]		6.53 (±0.58) [b]	4.37 (±0.29) [b]	2.16 (±0.24) [a]

[a] In the comparison of the same type of index, results having different letters are significantly different ($P < 0.05$).
[b] Control group 1: TFSP-based sausage prepared without canola oil.
[c] Control group 2: TFSP-based sausage prepared with direct-added canola oil and the sausage formulation is the same as TFSP-SPI14%-CO40%.

effectively immobilize both water and oil (Broucke et al., 2022; Lin & Barbut, 2025). By contrast, the incorporation of pre-formed SPI-CO emulsions markedly improved the WFB capacities of TFSP-based batters by significantly ($P < 0.05$) decreasing the TFL, WL, and FL compared to the control groups. For TFSP-SPI12-14%-CO20-40%, the TFL and WL showed a decreased tendency by increasing the SPI content and CO content in SPI-CO emulsions, while the FL remained essentially constant. The TFL, WL and FL of TFSP-SPI14%-CO40% were determined to be 3.31%, 2.92%, and 0.39%, respectively. This finding was consistent with Lee et al. (2023), who reported that the incorporation of pre-formed emulsions offered an approach not only to reduce the fat loss but also to reduce the water loss of plant-based meat products. Cao et al. (2022) reported that the filler effect of protein-stabilized emulsion droplets could reinforce gel network formation, thereby reducing the loss of fluid from the sausages. However, compared to TFSP-based sausages prepared with stable SPI-CO emulsions (TFSP-SPI12-14%-CO20-40%), those prepared with unstable SPI-CO emulsions (TFSP-SPI16%-CO20-40%) exhibited significantly ($P < 0.05$) higher TFL, WL, and FL. These results imply that the oil needs to be incorporated in the form of stable emulsion droplets which do not coalesce and flocculation, as otherwise this would result in liquid loss and poor quality.

3.4. Effects of pre-formed SPI-CO emulsions on the heat-induced gelation of TFSP-based sausages

In order to develop a better understanding of how pre-formed SPI-CO emulsions affect the gelling of TFSP-based sausages, the rheological behaviours of TFSP-based sausage matrix (batter) formulated with different SPI-CO emulsions during the temperature cycle of 25 °C→100 °C→25 °C were investigated. As shown in Fig. 5a, during the heating stage (25 °C→100 °C), the G' of control group 1 decreased slightly as the temperature increased from 25 to 51 °C, and then kept nearly constant between 51 and 100 °C; during the constant temperature holding stage (100 °C, 20 min), the G' increased slightly, and then started increasing progressively during the cooling stage (100 °C→25 °C). Similar changes in G' of batters prepared with only ground TVP during heating-cooling temperature cycle have been reported in literature, suggesting that the network formation observed in control group 1 would therefore most likely be derived from the gelling behaviours of TFSP (Lin & Barbut, 2025; Luo et al., 2024). In contrast, as shown in Fig. 5b–d, TFSP-based batters prepared with SPI-CO emulsions underwent a complex process of change during the heating stage: as the temperature increased from 25 to 63 °C, the G' decreased slightly, and then increased markedly as the temperature increased to ca. 80 °C; with the temperature increasing further from 80 to 100 °C, the G' decreased. These results indicated that the incorporation of SPI-CO emulsions significantly altered the gelling behaviours of TFSP-based batters. The initial decline in G' of TFSP-based batters might be attributed to the weakened physical interactions (e.g., hydrogen bonding, van der Waals effect) due to the increased temperature. However, the heat-induced gelling behaviours of emulsion-filled batters are not simply affected by strengthening or weakening of physical bonds but also by rearrangements occurring in the gel network (Jiang & Xiong, 2015). In fact, the TFSP-based batters formulated with SPI-CO emulsions showed a G' trajectory during temperature cycle similar to that of SPI-stabilized

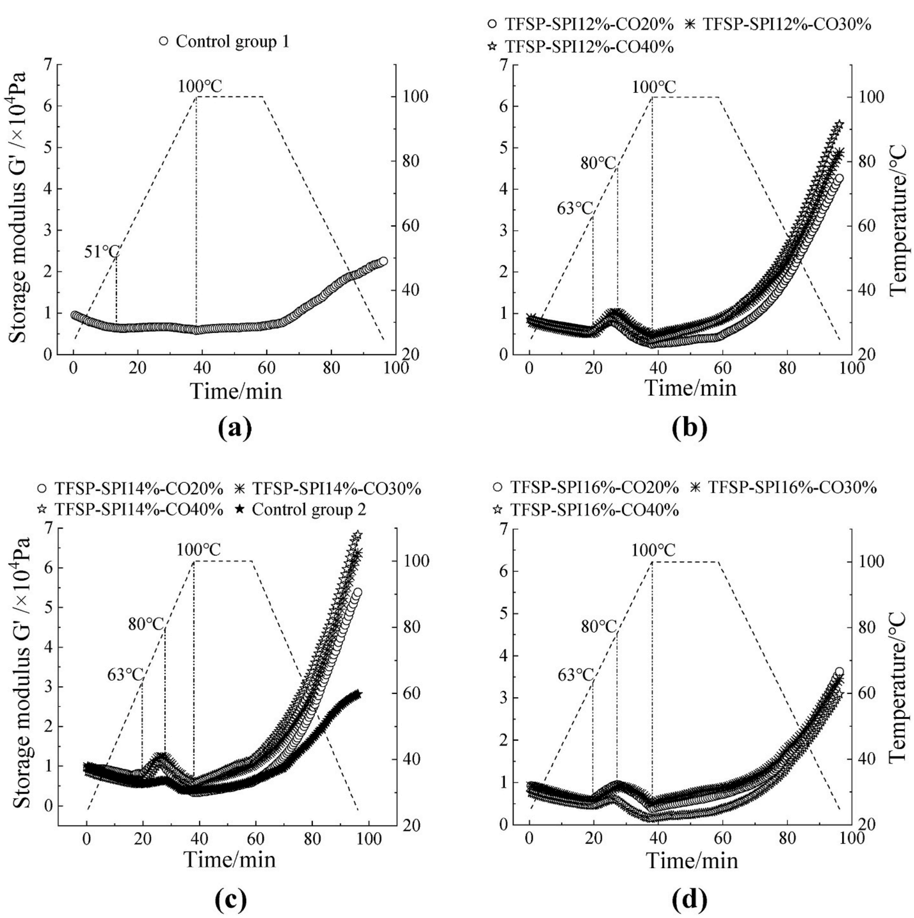

Fig. 5. Changes in storage modulus G' of TFSP-based sausage batters formulated with different SPI-CO emulsions during the temperature cycle (25 °C→100 °C→25 °C). (a) Control group 1: TFSP-based sausage prepared without canola oil; (b) TFSP-SPI12%-CO20-40%; (c) TFSP-SPI14%-CO20-40% and control group 2: TFSP-based sausage prepared with direct-added canola oil and the sausage formulation is the same as TFSP-SPI14%-CO40%; (d) TFSP-SPI16%-CO20-40%.

emulsion gels (Kim et al., 2001; Lin & Barbut, 2024, 2025). This finding suggests that not only SPI-CO emulsions played a role in binding different ingredients together, but also served as gelling agent participating the gel network formation in TFSP-based batters. During the constant temperature holding stage and the cooling stage, the *G'* progressively increased in all TFSP-SPI-CO batters, indicating that all batters became stiffer after the temperature cycle.

Fig. 6 shows the final *G′* values of different TFSP-based sausage batters. It is seen that the final *G′* values of TFSP-SPI12-14%-CO20-40% were significantly ($P < 0.05$) higher than those of control groups and increased with increasing SPI content and oil content. These results indicated that the incorporation of fine stable SPI-CO emulsions produced TFSP-based batters with pronouncedly enhanced gelling capability. However, with a further increase of SPI content up to 16 wt%, although an enhancement in viscosity was found for SPI16%-CO emulsion, a decrease in final *G′* values was observed for TFSP-SPI16%-CO sausage batters. The results of the final *G'* values of TFSP-based sausage batters are in good agreement with the results of TPA test, which might suggest that by using pre-emulsification technology, fine stable SPI-CO emulsions could be produced and incorporated as active fillers participating in the network formation to reinforce TFSP-based gel matrix. As a result, the gelling capability of TFSP-based batter were highly influenced by the technological characteristics of SPI-CO emulsions, such as emulsion viscosity and stability.

3.5. Effects of pre-formed SPI-CO emulsions on the microstructures of TFSP-based sausages

CLSM images of TFSP-based sausages formulated with different SPI-CO emulsions are shown in Fig. 7. The oily substance appears green, and the proteinaceous substance appears red in CLSM images. Control group 1 showed a loose and porous gel network consisting of small pieces of TFSP. The porous structure was characterized as black colour void spaces between the gel network, as also observed previously by Luo et al. and Lin et al. in microstructures of TVP-based gel matrix using CLSM (Lin & Barbut, 2025; Luo et al., 2024). This may be because TVP composed of protein aggregates possessed low protein solubility, and was less conducive to crosslink with each other and could only form a weak gel network (Xiao et al., 2025). Control group 2 appeared to contained a lot of big droplets and droplet flocs, indicating that the direct-added canola oil was not emulsified effectively. This finding confirms that it is not possible to produce a stable emulsion by simple

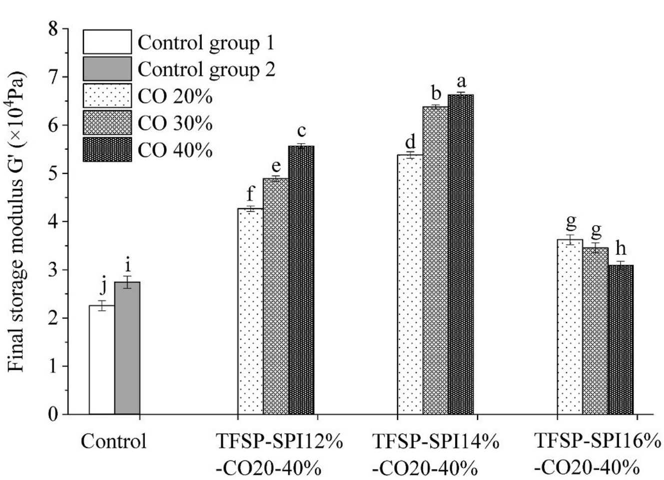

Fig. 6. The final storage modulus *G′* of TFSP-based sausage batters formulated with different SPI-CO emulsions after the temperature cycle (25 °C→100 °C→25 °C). Control group 1: TFSP-based sausage prepared without canola oil; Control group 2: TFSP-based sausage prepared with direct-added canola oil and the sausage formulation is the same as TFSP-SPI14%-CO40%.

and direct addition of liquid vegetable oil into TFSP-based sausage, because the extremely high viscosity of sausage batters technologically prohibits the effective emulsification of vegetable oil. The big droplets and droplet flocs distributed throughout but did not adhere to the sausage matrix, indicating that they had a weak affinity (i.e. the tendency of one thing to adhere to another) for TFSP pieces.

In contrast, For TFSP-SPI12-14%-CO20-40%, emulsion droplets appeared to be fine and adhered to the sausage matrix, indicating that they had a strong affinity for TFSP. It is observed that fine stable emulsion droplets helped to bind separate TFSP pieces together to form a TFSP-emulsion composite gel. And the increase of SPI content and CO content in SPI12-14%-CO20-40% was found to lead to an increasing gel firmness as area occupied by SPI-CO emulsions was increased. Luo et al. (2024) reported that emulsions containing small-size droplets reinforced the TFSP-based gel matrix more than did emulsions comprising large droplets, because the number of oil droplets increased as the droplet size decreased, thereby producing a greater total surface area to allow stronger droplet-protein network interaction. It should be noted that small-size droplets had highly curved interface, and when globular soy proteins adsorbed onto the droplet interface, their globular conformation would unfold due to the Laplace pressure force, which could be calculated based on Young-Laplace equation: $\Delta p = 2\gamma/a$, where Δp is the Laplace pressure (unit: Pa), γ is the interfacial tension (unit: mN/m), and a is the radius of droplet (unit: m) (Dickinson, 2009). According to our previous studies, the interfacial tension between canola oil-water interface at saturation coverage of SPI was ca. 20 mN/m (Chen et al., 2011). This implies a large pressure force of the order of 10^4 Pa for an SPI-stabilized oil droplet with a radius ≤4 μm. Moreover, it can be inferred from the Young-Laplace equation that the smaller the droplet is, the larger the Laplace pressure will be, and therefore the more unfolded protein conformation will be present at droplet interface (McClements & Gumus, 2016). Unfolding of globular soy proteins allowed the exposure of active groups (e.g., hydrophobic groups, sulfhydryl groups) previously buried, which would make the SPI stabilized droplets become more interactive with each other as well as with the sausage matrix. Therefore, not only a smaller droplet size provided more surface area, but also it caused an enhanced affinity between the interfacial proteins of emulsion droplets with the sausage matrix, a highly feasible explanation for the pronouncedly enhanced gelling capability of TFSP-based sausages prepared with fine stable SPI-CO emulsions (SPI12-14%-CO20-40%).

However, droplet flocculation caused a decrease in the affinity between emulsion droplets and sausage matrix formed by TFSP. As shown in Fig. 7, emulsion droplets appear to flocculate and disperse inhomogeneous in TFSP-SPI16%-CO20-40% sausages as those observed in SPI16%-CO20-40% emulsions (see Fig. 2). The gel matrix of TFSP-SPI16%-CO20-40% had a lot of void spaces, suggesting that the cross-linking between droplet flocs and gel matrix was weak. It should be noted that inadequately emulsification leads to the re-coalescence and bridging flocculation of emulsion droplets (Chen et al., 2018). Unlike depletion flocculation, which is caused by weak attraction between droplets, bridging flocculation was caused by the sharing of the adsorbed layer amongst adjacent droplets and was considered to be strong and irreversible aggregation (Dickinson, 2019). This means that bridging flocculation of emulsion droplets may lead to the burying of active groups of interfacial proteins, resulting in a decrease of their affinity for the sausage matrix. These observations are closely correlated with textural, WFB capacity and rheological measurements, where TFSP-based sausages prepared with unstable SPI-CO emulsions (SPI16%-CO20-40%) showed softer texture, poorer WFB capacities and lower heat-induced gelling capability compared to those prepared with stable SPI-CO emulsions (SPI12-14%-CO20-40%).

4. Conclusion

To summarize, this work demonstrated that at SPI contents = 12–14

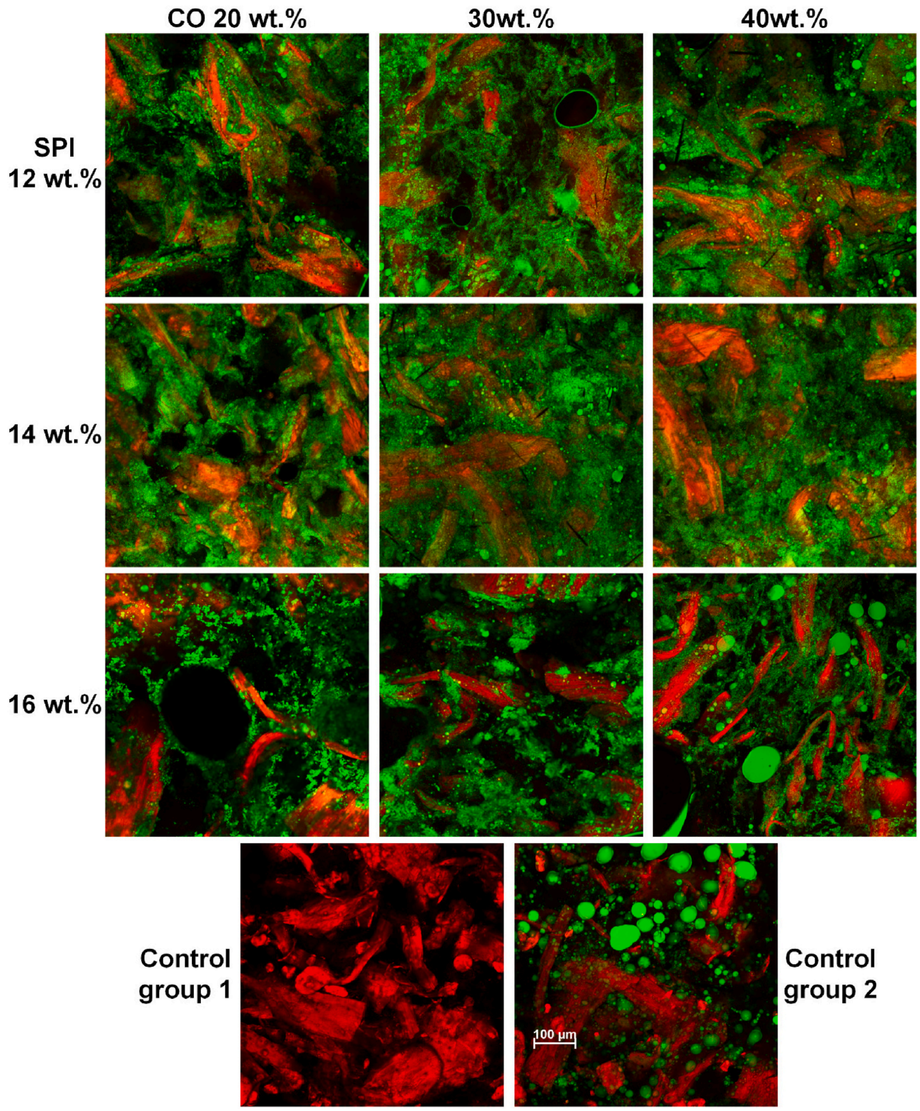

Fig. 7. CLSM images of TFSP-based sausages formulated with different SPI-CO emulsions. Control group 1: TFSP-based sausage prepared without canola oil; Control group 2: TFSP-based sausage prepared with direct-added canola oil and the sausage formulation is the same as TFSP-SPI14%-CO40%. Bar = 100 μm.

wt% and CO contents = 20–40 wt%, stable SPI-CO emulsions (no sign of droplet flocculation) with small droplet sizes (d_{43} = 2.85–6.94 μm) could be formed, and showed an increased binding ability by increasing SPI content and CO content. When SPI12-14%-CO20-40% emulsions were incorporated into TFSP-based sausages, find stable emulsion droplets showed a strong affinity for TFSP, and behaved as active filler binding separate TFSP pieces together to form a TFSP-emulsion composite gel. This may be due to the fact that when SPI adsorbed onto the highly curved droplet interfaces, the globular protein conformation will unfold due to the Laplace pressure force of large magnitude, which made SPI-stabilized emulsion droplets become more interactive with each other as well as with the TFSP. As a results, the heat-induced gelling capability of TFSP-based sausage matrix was markedly enhanced, thereby producing prominent benefits in improving textural properties and WFB capacities of final sausage products. However, the use of excessive SPI (16 wt%) and/or CO (50 wt%) led to fast coalescence and bridging flocculation of droplets during emulsification, which caused a drastic decrease in the affinity of emulsion droplets for TFSP-based sausage matrix.

In conclusion, TFSP-based sausages could be an excellent alternative of real meat products with oil pre-emulsification technology leading to desirable modification on texture and quality characteristics of plant-based meat products. However, further studies are needed to elucidate more clearly the details of the mechanism involved the changes in protein conformation of SPI after adsorbing onto droplet surface that make it become more interactive with TFSP.

CRediT authorship contribution statement

Lin Chen: Writing – review & editing, Writing – original draft, Validation, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Lin Yu:** Writing – review & editing, Supervision, Resources, Project administration, Investigation, Formal analysis, Conceptualization. **Wenjing Ji:** Writing – review & editing, Validation, Resources, Methodology, Investigation. **Rammile Ettelaie:** Writing – review & editing, Validation, Methodology, Formal analysis, Conceptualization. **Zixuan Cheng:** Writing – review & editing, Visualization, Validation, Data curation. **Suhua Zhang:** Visualization, Validation, Data curation.

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.

Acknowledgement

We are grateful for the financial support from National Natural Science Foundation of China (No. 32372271) and Natural Science Foundation of Guangdong Province (No. 2023A1515011563). LC acknowledges the financial support from the School of Chemical Engineering and Light Industry for his studies in the University of Leeds.

Data availability

Data will be made available on request.

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