

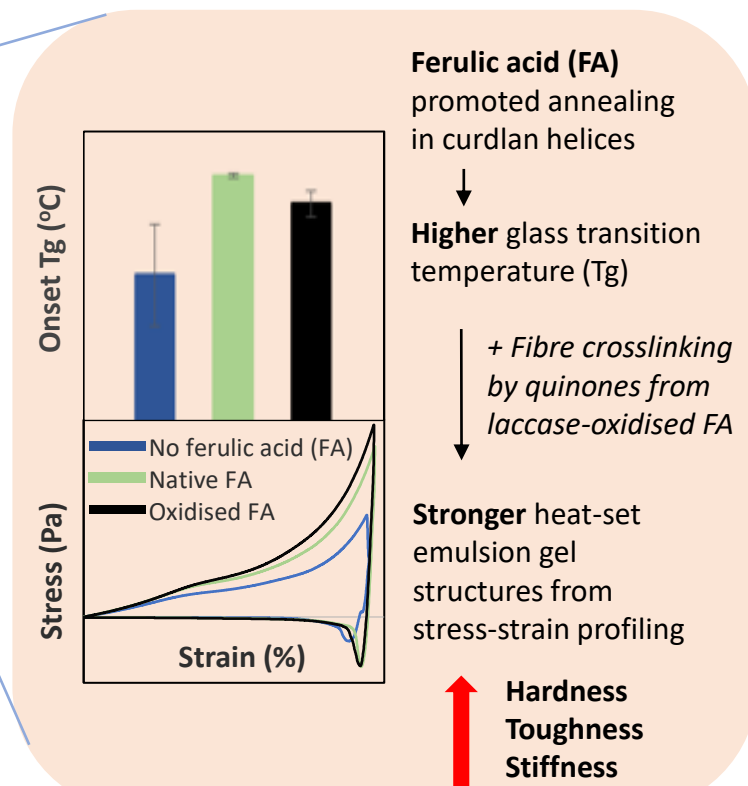
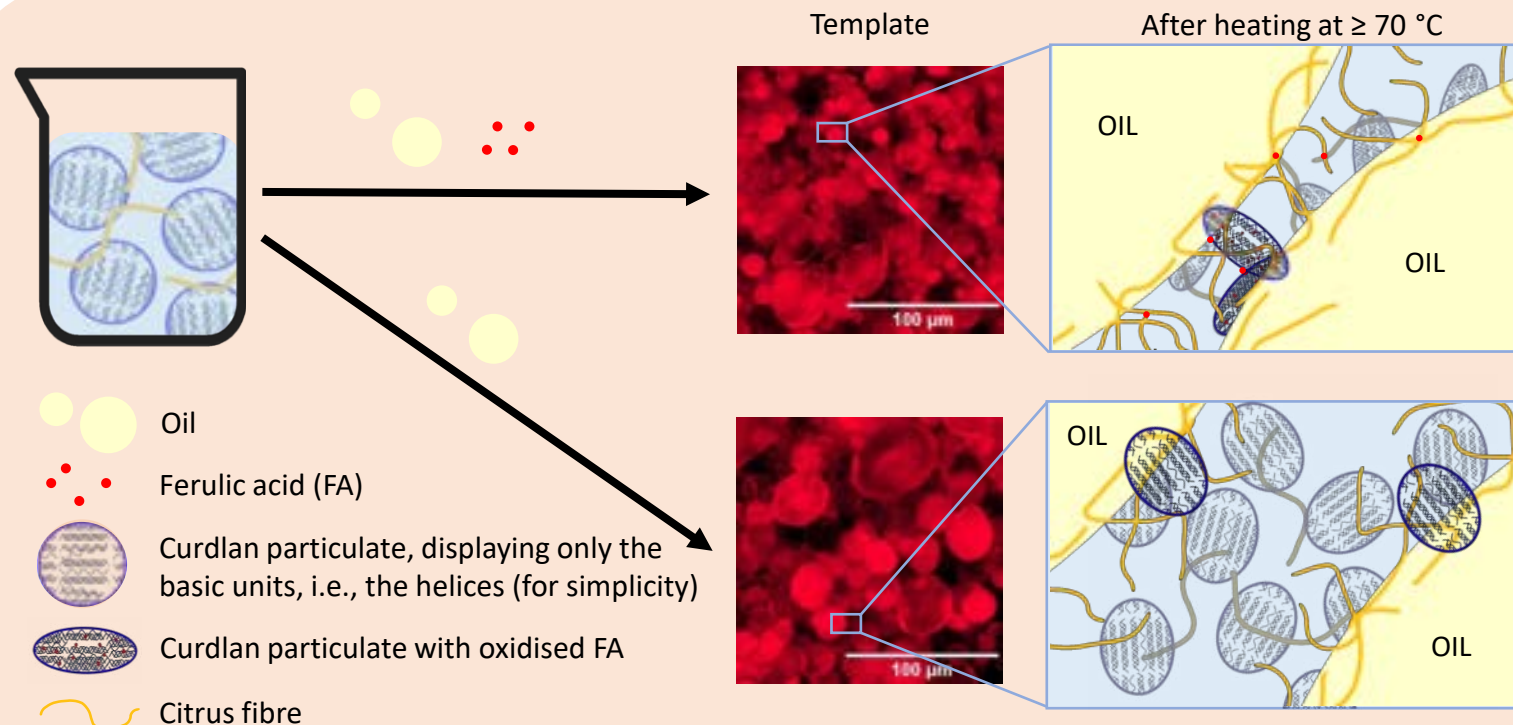
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New insights into the strengthening of fibre-based emulsion gels by phenolic compounds

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Abstract:	Consumer demand for plant-based meat alternatives and health concerns over animal fats have propelled the exploration of edible oil structuring techniques, for mimicking animal fat texture and functionality. In this context, the use of food polymers offers possibilities in conferring viscoelastic properties to oil structured in heterogeneous systems. Many reported examples were predominantly structured by proteins, however, which are sensitive to ionic strength. In this work, we offer an alternative approach through emulsion gel structuring using curdlan and citrus fibre, which are natural and more process-stable food polymers. This work reports insights gained on emulsion gel strengthening with the use of laccase-oxidised phenolic compounds. Our key results reveal the use of laccase-oxidised ferulic acid at millimolar concentration against 10% (w/w) curdlan to be particularly promising, in that (i) it strengthened inter-triplex network in heat-set curdlan, as supported by the increase in glass transition temperature of curdlan; (ii) it generated quinones as determined by nitro blue tetrazolium-glycinate assay, which could crosslink interfacial fibre having trace nitrogen; and (iii) consequently, the emulsion gel system became stronger against high strain compression. Crosslinking at inter-oil-droplet bridges with well-controlled oil aggregate sizes was pivotal, and this could be optimised with suitable sequencing of formulation steps that keep the aqueous phase viscosity low during emulsification. Overall, our findings provide reference value in the creation of stiff animal fat mimetics out of unsaturated edible oils.

New insights into the strengthening of fibre-based emulsion gels by phenolic compounds



Highlights

- Ferulic acid (FA) strengthened the inter-triplex network in heat-set curdlan
- Curdlan was found present at the oil-water interface in fibre-based emulsion gel
- Quinones of laccase-oxidised FA crosslinked interfacial fibre having trace nitrogen
- Put together, laccase-oxidised FA strengthened emulsion gel against compression
- Other key factors were low viscosity during emulsification and small oil aggregates

Title

New insights into the strengthening of fibre-based emulsion gels by phenolic compounds

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Abstract

Consumer demand for plant-based meat alternatives and health concerns over animal fats have propelled the exploration of edible oil structuring techniques, for mimicking animal fat texture and functionality. In this context, the use of food polymers offers possibilities in conferring viscoelastic properties to oil structured in heterogeneous systems. Many reported examples were predominantly structured by proteins, however, which are sensitive to ionic strength. In this work, we offer an alternative approach through emulsion gel structuring using curdlan and citrus fibre, which are natural and more process-stable food polymers. This work reports insights gained on emulsion gel strengthening with the use of laccase-oxidised phenolic compounds. Our key results reveal the use of laccase-oxidised ferulic acid at millimolar concentration against 10% (w/w) curdlan to be particularly promising, in that (i) it strengthened inter-triplex network in heat-set curdlan, as supported by the increase in glass transition temperature of curdlan; (ii) it generated quinones as determined by nitro blue tetrazolium-glycinate assay, which could crosslink interfacial fibre having trace nitrogen; and (iii) consequently, the emulsion gel system became stronger against high strain compression. Crosslinking at inter-oil-droplet bridges with well-controlled oil aggregate sizes was pivotal, and this could be optimised with suitable sequencing of formulation steps that keep the aqueous phase viscosity low during emulsification. Overall, our findings provide reference value in the creation of stiff animal fat mimetics out of unsaturated edible oils.

Keywords: Emulgel; plant-based fat; phenolic acid; laccase; curdlan; lipid structuring

1. Introduction

With the mega-trend of healthier, sustainably produced plant-based meat, methods for formulating animal fat mimetics for high fat meat analogues are in increasing demand, partly to reduce cholesterol and saturated fat intake as advocated by World Health Organisation (*Saturated fatty acid and trans-fatty acid intake for adults and children: WHO guideline*, 2023), and partly to overcome consumer aversion by delivering more textural authenticity (Kolodziejczak et al., 2021). For a convincing animal mimetic, it goes beyond rendering edible fat in a solid(-like) form to simulate fat crystal networks or aggregates with entrapped liquid oil (Acevedo & Marangoni, 2010). More importantly, there needs to be viscoelasticity (Gómez-Guillén et al., 2011) as found in animal fat tissue with fat crystals incorporated within collagenous connective tissue (Dreher et al., 2020; Wood et al., 2004). Conceivably, contrary to using lipid-based gelators, assembling oil in a heterogeneous system

comprising food polymers offers more structuring opportunities, and thus a more possible route to endow higher bulk elastic modulus (G') at relevant food serving temperatures.

As with all heterogeneous systems in general, the bulk strength of a structured oil may be modulated by different mechanisms of interfacial bonding. Two related examples are a binary solid-in-liquid system and a ternary solid-liquid-liquid system, whereby the solids are in a three-dimensional, well-suspended aggregate form. As an example of the former, submicron-sized aggregates of denatured protein could form a relatively stronger gel network in oil when solid-oil interaction was poorer. However, a possible disadvantage with the network is its high shear-sensitivity (de Vries et al., 2017). In the latter, apart from an oil continuous phase and micronised solid particles, there is an aqueous secondary liquid which could either be in a dispersed phase (Lee et al., 2012) or form interparticle capillary bridges to hold the structure in place (Calabrese et al., 2021). Calabrese et al. (2021) tested with pea hull and pea epidermal cell particles and found that at optimal volume fractions of the particles and aqueous phase, solid-like bulk properties such as a paste-like appearance could be attained. To strengthen the aqueous bridges and in turn the bulk system, gelation via cross-linkable polymers could be induced (Huang & Berg, 2003), so long as microscopic phase separation does not set in (Yang et al., 2020).

In another example, oil structuring stems from aggregated droplets of oil itself, held together by electrostatic attraction due to opposite and sufficiently high surface charges. This mechanism is called heteroaggregation, for which the surface charges could come from protein coatings with different isoelectric points at the pH in between, or other pairings of oppositely charged emulsifiers. In the case with protein coatings, the inter-droplet attraction could be enhanced by hydrophobic interaction from heat-induced protein denaturation, bringing about higher bulk elastic or complex modulus in general. However, depending on the protein type and the proportion of positively and negatively charged droplets, the system could be susceptible to collapse upon cooling (Li & McClements, 2023; Mao & McClements, 2012). Structural dissociation during dilution and shearing had also been reported for aggregates of oil droplets stabilised only electrostatically by saponins and the emulsifier sodium-lauroyl-L-arginine ethyl ester (Maier et al., 2014). To alleviate the risk of fragility in a heteroaggregate, mixed emulsion system stabilised by negatively charged Quillaja saponins and positively charged proteins, Maier et al. (2015) resorted to laccase-induced covalent crosslinking to yield an oil-entrapping protein network. The efficacy of the mechanical strengthening depended on the protein reactivity with electrophilic quinones, formed from the deprotonation of phenoxy radicals which in turn were derived from laccase-catalysed one-electron oxidation of phenolic compounds (Asmus & Bonifačić, 2000) inherently present in the saponins. Specifically, monomer or oligomeric forms of the quinones could undergo Michael addition or Schiff base reactions with nucleophilic amines to form

covalent adducts (Adelakun et al., 2012; Kudanga et al., 2011). This could occur from as low as 15°C based on second-order rate constants (Liu et al., 2023).

In the above cited oil aggregate systems with bulk elastic properties, reportedly successful examples were few and heavily reliant on protein, which makes them highly sensitive to ionic strength. Viscoelastic, structured food lipids that are ionic strength resistive are hardly known. To fill this gap, we built on our recent work in emulsion gel hybrids structured by insoluble fibres, namely the elastic curdlan and emulsifying, high shear-activated citrus fibre (Tan & Phoon, 2023). This time, we directed our focus on enhancing structuring with fibres by phenolic compounds. Priorly, we found via the Dumas method that at the total fibre percentage used, there was concomitantly 0.17% (w/w) nitrogen content on average in the emulsion gel system. This trace nitrogen content could be ascribed to proteinaceous groups along the main citrus fibre chain (Lundberg et al., 2014; Nakamura et al., 2004) and nitrogen content from the microbial fermentation broth during commercial curdlan production (Saudagar & Singhal, 2004; Wang et al., 2016). While this work is inspired by phenolic compound-polymer grafting, especially laccase-mediated crosslinking of nitrogen-rich polymers with either phenolic acids or intrinsically feruloylated carbohydrates (e.g., pectin and arabinoxylans) (Deng et al., 2018; Robert et al., 2022; Sakai et al., 2021), it abides to a key principle of working only with meagre nitrogen content. In this work, the crosslinking action of exogenous, food grade phenolic compounds subjected to laccase-oxidised was tested. The tested phenolic compounds were of varying molecular weight and polarity, namely, ferulic acid (phenolic acid, MW: 194.06 g/mol), epigallocatechin gallate (flavonoid, MW: 290.08 g/mol), and ortho-proanthocyanidin (flavonoid, MW B2: 576.5 g/mol) extracted from grape seed.

The highlights and novelty of this work lie in discovering the art in strengthening the fibre-based emulsion gel system by exogenous phenolic compounds. As the reader would find out, not all phenolic compounds worked the same and for the same constituents at the same percentages, the elastic modulus (G') could still be different. This work presents unique perspectives underlying the bulk strength of a fibre-based emulsion gel system. Specifically, this paper reports the physical influence of laccase-oxidised phenolic compounds on the molecular configuration and inter-helix structure of curdlan, which is crucial due to its role as the structuring fibre that confers mechanical strength. It pinpoints how quinone-driven crosslinking of fibres at the oil-water interface is more influential than gelation in the continuous aqueous phase. It underscores the importance of sequencing in the oil-structuring process, and the inverse correlation between bulk strength and the size of oil aggregates.

2. Materials and materials

100 **2.1. Materials**

101 Commercial citrus fibre (CFN) was received from CP Kelco (Atlanta, Georgia, U.S.) and curdlan was
 102 purchased from Natural Colloids Industries Pte. Ltd. (Singapore). Sunflower oil with 85% (w/w) average
 103 total unsaturated fat content and pork fat tissue were bought from a local supermarket. All food-grade
 104 chemicals and their sources were as follows: 32% (w/w) hydrochloric acid (HCl) (Sigma-Aldrich, St.
 105 Louis, Missouri, U.S.); caprylic triglyceride (MCT) from MegaChem Pte. Ltd. (Singapore); (EGCG) from
 106 Henan Alfa Chemical Co. Ltd. (Zhengzhou, Henan, China); ferulic acid (FA) from Penta International
 107 Corporation (West Caldwell, New Jersey, U.S.); grape seed extract derived ortho-proanthocyanidin
 108 (OPC) from Wellgreen Technology Co. Ltd. (Xi'an, Shaanxi, China); laccase powder with 10000 U/g
 109 activity (from *Aspergillus oryzae*) from Sunson Industry Group Co. Ltd. (Yinchuan, Ningxia, China);
 110 polyglutamic acid (PGA) from Shandong Freda Biotechnology Co. Ltd. (Linshu Linyi, Shandong, China);
 111 sodium hydroxide (NaOH) from Fisher Scientific (Pittsburgh, Pennsylvania, U.S.). All chemical reagents
 112 were from companies as follows: 200 proof ethanol from Pharmaco (Brookfield, Connecticut, U.S.);
 113 acetonitrile, Direct red 23, formic acid, glycine, nitrotetrazolium blue chloride (NBT), and potassium
 114 hydroxide from Sigma-Aldrich (St. Louis, Missouri, U.S.); Nile Red fluorescent dye from Santa Cruz
 115 Biotechnology, Inc. (Santa Cruz, California, U.S.). Sodium 8-anilino-1-naphthalenesulfonate (ANS-Na)
 116 fluorescent dye was bought from Tokyo Chemical Industry Co., Ltd. (Tokyo Japan). Distilled water was
 117 used for the preparation of all samples.

118 **2.2. Liquid chromatography-mass spectrometry (LC-MS)**

119 LC-MS was done to verify laccase-catalysed oxidation of phenolic compounds. Based on known MW
 120 values of FA, EGCG, and OPC B2 which is naturally found in grape seed extract (Nakamura et al., 2003),
 121 1-4 mM aqueous solutions of phenolic compounds were prepared, with pH adjusted to 4.5-5.0 using
 122 only diluted HCl and NaOH. To trigger enzymic catalysis for every 100 g of solution, 0.34 g of laccase
 123 powder was dispersed in. With or without laccase, the phenolic compound solutions were incubated
 124 for 16 h at 45 °C in the dark. A negative control of laccase solution without phenolic compound was
 125 also prepared. The solutions were injected into an Agilent UHPLC 1290 Infinity unit connected to an
 126 Agilent 6540 quadrupole time-of-flight (QTOF) mass spectrometer, equipped with an electron spray
 127 ionization (ESI) source. A 2.1 × 50 mm HSS T3 column with 1.8 µm pore size was used under conditions
 128 as such: (i) flow rate of 0.4 mL/min; (ii) solvent A comprising water with 0.1% (v/v) formic acid; (iii)
 129 solvent B comprising acetonitrile + 0.1 % (v/v) formic acid; (iv) gradient conditions starting with
 130 isocratic solvent A for 0.5 min, 0-50 % solvent B over 4.5 min, 50-100% solvent B over 2 min, and lastly
 131 isocratic solvent B over 2 min.

2.3. Preparation of emulsion gel hybrid templates and their respective aqueous phase

Emulsion gel hybrid templates and their respective aqueous phase were prepared based on modifications from the methodology outlined in our previous study (Tan & Phoon, 2023). The term template refers to the bulk microstructure before the emulsion gel was heat-set. In this current study, the oil load was sunflower oil set at 50% (w/w) unless stated, and the curdlan concentration in the aqueous phase at 10% (w/w). A T25 Ultra-Turrax® disperser (IKA-Werke, Staufen, Germany) was used to shear-mix the samples at ≥ 6000 rpm during their preparation. Sample variations included substituting water as the aqueous medium with phenolic compound solutions either oxidised through laccase catalysis or not (see Section 2.2). In all samples containing laccase, the enzyme activity was 300 U per gram of curdlan. Four different sequences for sample preparation were also tested, in terms of the time points at which CFN, curdlan, and oil were incorporated and when the 16 h-incubation occurred. In sequence A, laccase was added after the emulsion gel structure was formed. In sequence B, incubation of the CFN-curdlan aqueous phase with laccase took place before oil was added and emulsified. In sequence C, incubation of fibre-free aqueous medium occurred; CFN was then homogenised in the aqueous medium and shear-mixed with curdlan before oil was added and emulsified. Sequence D was similar as sequence C except that the steps of shear-mixing in curdlan and oil were reversed. In further minor variations to sequences C and D, as tested in select FA-based emulsion gel hybrid templates, polyglutamic acid (PGA) was added together with curdlan before emulsification (as in sequence C) or after (as in sequence D). PGA was added at 0.2% or 0.4% (w/w) of the total aqueous phase to increase the nitrogenous content paltrily.

2.4. Solid-state ^{13}C cross polarisation magic angle spinning NMR spectroscopy (CP/MAS ^{13}C -NMR)

Solid-state ^{13}C CP/MAS NMR spectroscopy was used as a first probe for differential binding of oxidised phenolic compounds for curdlan under different circumstances of laccase catalysis (sequences A/B versus C/D). The tested phenolic compounds were FA, EGCG, and OPC. In sequence A/B, 1 mM phenolic compound was oxidised by 0.34% (w/w) laccase in 10% aqueous curdlan dispersion at 45 °C for 16 h in the dark. In sequence C/D, 1 mM phenolic compound was pre-oxidised by laccase at 45 °C for 16 h in the dark, before curdlan was mixed in at 10% (w/w). Mixing with curdlan was done using the Ultra-Turrax® disperser at ≥ 6000 rpm at room temperature. The mixtures were frozen at -70 °C overnight and freeze-dried in a Telstar LyoQuest -85 plus benchtop freeze dryer (Azbil Telstar, Terrassa, Spain). Then, approximately 100 mg of freeze-dried sample was analysed per run on an Avance NEO 400 MHz spectrometer (Billerica, Massachusetts, U.S.), with full sample loading on a 4 mm ZrO₂ CPMAS rotor and operation at a ^{13}C frequency of 100.64 MHz. Five hundred and twelve scans were

accumulated for each spectrum with a recycle delay of 5 s. The chemical shifts were referenced to tetramethylsilane (TMS) using adamantane as an external standard.

2.5. Rheological measurement

For each sample, duplicate runs were carried out on a MCR 302 rheometer controlled by a RheoCompass software (Anton Paar GmbH, Graz, Germany) on day 0 of sample preparation. A parallel plate geometry with a diameter of 19.965 mm was used at 1 mm sample gap. The samples were measured within 1 day of chilled storage in an ice bath (to inactivate any laccase present). Temperature ramping was conducted at rates that faintly resemble fast cooking on a hot pan and cooling in air.

2.5.1. One-cycle temperature sweep

Curdlan dispersions, emulsion gel hybrid templates, and their respective aqueous phase were rapidly heated from 20 °C to 95 °C at 38°C/min and then cooled back to 20 °C at 11 °C/min, at 0.2% strain and 1 Hz. This protocol allows a first quick comparison of the strength of various heat-set samples, through G' readings taken at 20 °C after cooling. A high heating rate of 38°C/min was within the operational capability of the rheometer, although it deviated from the real-life heating of emulsion gel templates on a hot pan, which was found to be as rapid as 60 °C/min.

2.5.2. Dual-cycle temperature sweep

Emulsion gel hybrid templates prepared according to Sequence D with FA and EGCG (at 1 mM aqueous concentration), with and without laccase-catalysed oxidation, were tested against a control without any phenolic compound. 10% (w/w) curdlan dispersions with and without CFN were also measured. G' and loss modulus (G'') were read at 0.2% strain and 1 Hz in two heat-cool cycles. The first cycle included a heating ramp from 20 °C to 70 °C at 25°C/min, holding at 70 °C for 1 min, followed by cooling from 70 °C to 20 °C at 10 °C/min and subsequent holding for 1 min. This was immediately followed by the second cycle, with a slower heating ramp from 20 °C to 100 °C at 10°C/min, holding at 100 °C for 1 min, and cooling from 100 °C to 20 °C at 10 °C/min. From the G' versus temperature plots, the hysteresis area enclosed by the second heat-cool curves within 20-60 °C were derived using OriginPro 9.4 (OriginLab, Northampton, U.S.) and compared. Incidentally, it was with the intention to evaluate sample thermal behaviour more closely, that the first heating rate was changed from of 38 °C/min to 25 °C/min. However, the change did not cause any impact on the sample G' .

2.6. Differential scanning calorimetry (DSC) assay

DSC was used to gain further insight on curdlan-phenolic compound interaction, specifically FA. Two batches of freeze-dried samples were prepared from 10% (w/w) curdlan suspensions without FA, with

1 mM native FA, or with 1 mM laccase-oxidised FA as per sequence A/B in Section 2.4. For batch 1, the suspensions were incubated for 16 h at 45 °C in the dark, then immediately frozen overnight at -70 °C and eventually freeze-dried. For batch 2, there was an additional step of heat-setting the suspensions at 70 °C for 10 min after the 16 h-incubation. The freeze-dried samples were run in duplicates on a DSC 214 Nevio unit (NETZSCH, Selb, Germany). Each sample run had 3.4-6.8 mg of mass loaded in an aluminium crucible pan with a non-pierced lid, and heat ramped from 180 °C to 300 °C at 5 °C/min to detect its glass transition temperature (T_g). The onset of T_g was analysed using the Proteus Analysis software (version 8).

2.7. Epifluorescence microscopy

Epifluorescence microscopy was performed on an Olympus BX53 upright microscope, with images captured by an Olympus microscope digital camera DP74 connected to a cellSens software (Olympus, Tokyo, Japan). Micrographs were adjusted in contrast and brightness using the image processing software Fiji.

2.7.1. For aggregate size measurement in emulsion gel hybrid templates

0.5 mL of each emulsion gel hybrid template of interest was stained with 5 µL of 0.015% (w/w) Nile Red dye in ethanol. Images from the use of a TRITC fluorescence filter were converted to binary images in Fiji using minimal and standardised procedural settings. Thereafter the aggregate size of oil droplets was analysed by averaging the diameters of the four largest aggregates.

2.7.2 For imaging of interfacial fibre separated from emulsion gel hybrid templates

Emulsion gel hybrid templates with 1 mM of native or laccase-oxidised FA in aqueous phase and 40% (w/w) caprylic triglyceride oil load were prepared according to sequence D. Each sample was centrifuged at 20,000 rcf for 15 minutes at 20 °C, forming an interfacial fibre layer sandwiched between a top oil layer and the bottom emulsion gel mass. Using a needle syringe, the interfacial fibre layer was extracted into a 1.5 mL eppendorf tube, washed with distilled water (solvent) at 1:1 mass ratio through vortex-mixing, and centrifuged again under the same conditions. The solvent was removed using a needle syringe. The retained interfacial fibre was washed a second time in a repeat cycle. The final retained interfacial fibre was diluted with distilled water at a 1:1 mass ratio and left standing for sedimentation. An aliquot of 10 µL from each sediment was co-stained by 5 µL of 1% ANS-Na in ethanol and 5 µL of 0.75% Direct red 23 in distilled water on a microscopy slide, and detected for presence of curdlan and citrus fibre using DAPI and TRITC fluorescence filters.

2.8. Nitro blue Tetrazolium (NBT)-glycinate redox staining assay

NBT-glycinate redox staining assay is a technique widely used to detect quinones, through the ability of them or their adducts with amine groups to catalyse the reduction of yellow NBT to a blue-back formazan by excess glycine at alkaline pH (Paz et al., 1991). The assay was performed first in Section 2.8.1 to confirm its higher sensitivity to laccase-oxidised FA over native FA, and finally in Section 2.8.2 to determine if interfacial fibre could be involved in laccase-catalysed crosslinking.

Section 2.8.1. Sensitivity of assay to laccase-oxidised ferulic acid

Selected freeze-dried, non-heat-set curdlan samples from Sections 2.4 and 2.6, namely (1) curdlan alone, (2) curdlan with 1 mM native FA, (3) curdlan with laccase-oxidised FA prepared according to sequence A/B, and (4) curdlan with laccase-oxidised FA prepared according to sequence C/D, were analysed. To each sample, a 0.41 mM solution of NBT in 2 M potassium glycinate (pH 11) was added at 1:10 mass ratio. For their corresponding controls, 2 M potassium glycinate (pH 11) was added instead. All samples and controls were incubated in the dark at room temperature for 5, 45, and 90 min. At each time, photographs were taken under the same light condition and camera settings.

Section 2.8.2. Detection of potential laccase-catalysed crosslinking in interfacial fibre

To aliquots of the re-dispersed, diluted interfacial fibre samples (before sedimentation) and the washing solvents collected from Section 2.7.2, 0.41 mM NBT solution in 2 M potassium glycinate (pH 11) was added to each at 1:4 mass ratio. The mixtures were incubated in the dark at room temperature for 45 minutes. A negative control being 0.41 mM NBT in 2 M potassium glycinate (pH 11) alone was also kept in the dark at room temperature for this long. After incubation, the samples were vortexed and read at 560 nm in 150 μ L volumes in a 96-well clear plate with flat bottom, using a Cytation 5 Cell Imaging Multi-Mode Reader (BioTek, Winooski, Vermont, U.S.).

2.9. Stress-compressive strain profiling and textural measurement of heat-set emulsion gels

The experiment was done on day 0 of sample preparation. Emulsion gel hybrid templates which were tested in Section 2.5.2 were steamed for 10 min and then cooled to ambient temperature. Block-shaped samples of the heat-set emulsion gels, measuring $2.0 \times 2.0 \times 0.85$ cm (width, length, height), were used for stress-strain profiling on a TA.XT.plus Texture Analyser (Stable Micro Systems, Surrey, U.K) at 80% compressive strain. A P/50 cylindrical probe was used for the test. The trigger force was set at 2g while all test speeds were set of 1.0 mm/s. Using an Exponent Connect (version 8.1.2.0) software with existing and self-written macro functions, average stress-strain curve profiles (n=3) and average values of toughness, hardness, and change in stiffness under high deformation were obtained (n=5). Specifically, toughness was determined by the positive area under the compression curve between 0% and 75% strain. For standardisation, hardness was reported as the force to achieve 75% compressive

strain, which was or very close to the peak force for each sample. Lastly, change in stiffness was taken from 60% to 70% compressive strain.

2.10. Statistical analysis

An online one-way analysis of variance (ANOVA) with post-hoc Tukey test calculator (Vasavada, 2016) was used to perform statistical analysis at $n \geq 2$. The data analysed were the hysteresis area results generated in Section 2.5.2, onset values of Tg from Section 2.6, NBT absorbance readings from Section 2.8, and stress-strain profiling results obtained in Section 2.9.

3. Results and discussion

3.1. Assessment of laccase-catalysed oxidation of phenolic compounds

Fig. 1 shows the LC-MS base peak chromatograms (BPC) obtained in negative ion mode for native FA, EGCG, and OPC (Figs 1A, 1C, and 1E), oxidised FA, EGCG, and OPC following 16 h incubation with laccase (Figs 1B, 1D, and 1F), and laccase alone as a control (Fig. 1G). The analyte concentration chosen for each phenolic compound, as stated in the figure caption, was the highest concentration worked with for subsequent discussion below. The LC-MS method used might not be sensitive for quinone detection, due to lack of prior derivatisation step (Pei et al., 2018) and due to the acidic condition of the mobile phase which could promote quinone reduction. While separate discussion on quinone detection would be covered in Section 3.5, Fig. 1 provides a first glimpse on laccase-catalysed oxidation of the phenolic compounds. In general, the polarity of FA (native, oxidised) was lower than that of the other phenolic compounds. During laccase-catalysed oxidation of FA, there was significant increase in species with ion peaks [M-H]⁻ of m/z 341.1030 and m/z 385.09297. M/z 385.0929 represents the dimer of ferulic acid, which was putatively identified as a mixture of 3 compounds, 8-5'-Benzofuran-diferulic acid, trans-5-[(E)-2-carboxyvinyl]-2-(4-hydroxy-3-methoxy-phenyl)-7-methoxy-2,3 dihydrobenzofuran-3-carboxylic acid, and 7,8-cis-8-8-y-Lactone-diferulic acid. M/z 341.1030 represents residual compounds formed after further dissociation of a carboxylic group (Carunchio et al., 2001). The oxidised products of FA eluted later than native FA, suggesting their lower polarity. In the case of EGCG, laccase-catalysed oxidation led to a reaction product with ion peaks [M-H]⁻ of m/z 761.1367 and m/z 913.1464. m/z 761.1367 was putatively identified as theasinensin B, which is the dimer of EGCG and epicallocatechin, while m/z 913.1464 was putatively identified as theasinensin A, which is the dimer of EGCG (Chen et al., 2016). There were few earlier elution peaks in the oxidised products of EGCG than native EGCG, suggesting their higher polarity. 1E and 1F appear similar, with the species from the

acquisition time of 2.8 to 3.6 min found to include both procyanidin B2 and its converted form proanthocyanidin A2. Due to this similarity, the OPC ingredient used was believed to have already oxidised during its mass commercial production.

To understand if the imposed conditions of laccase catalysis had conformational implication on fibre which underlies the emulsion gel structure, comparison of ¹³C NMR spectra of the structuring fibre curdlan would be beneficial. Curdlan has five pronounced chemical shift peaks in its ¹³C NMR spectrum when anhydrous (Saitô et al., 1998). C-3, with a chemical shift around 89.9 ppm (Saitô et al., 1998), is one of the interior-facing carbons in its helix in closer atomic proximity (Cai & Zhang, 2021; Yan et al., 2021). Due to the mild conditions of sample preparation as per Section 2.4, with temperature never above 45 °C, it was expected that bundles of the curdlan triple-stranded helices would at most swell under hydration (prior to freeze-drying) (Xiao et al., 2017) but not dissociate to create increased accessibility of their hydrophobic cavities. In this case, any binding with oxidated phenolic compounds is likely more hydrophilic than hydrophobic in nature. For the shown ¹³C NMR spectra of anhydrous curdlan in Fig. 2, the ones associated with sequence A/B during sample preparation had curdlan present as laccase-catalysed oxidation evolved over 16 h, while the ones associated with sequence C/D had curdlan added only after 16 h of prior oxidation. Thus, by referencing Fig. 1, the FA oxidation products which curdlan had exposure to in Fig. 2A (ii) should overall be more hydrophobic than those in Fig. 2A (i), while the EGCG oxidation products in Fig. 2B (ii) should generally be more hydrophilic than those in Fig. 2B (i). From scrutinising Fig. 2A, a minor upfield peak in C-3 chemical shift at 86.0 ppm was found in (i), indicative of binding of less hydrophobic FA oxidative products to curdlan that led to a molecular configuration with a different torsion angle and a C-3 nucleus more shielded in electron density. The same minor peak was also found in Fig. 2B (ii), indicative of binding of more hydrophilic EGCG oxidative products to curdlan. The corresponding spectra for OPC are omitted in Fig. 2 due to their similarity, which reinforces the belief that the OPC ingredient used had already oxidised during its mass production.

Together, Figs. 1 and 2 only give insight to changes at ≤ 45 °C and are inconclusive of what happens with heat-setting of actual emulsion gel templates with oxidised FA and EGCG. Nonetheless, Figs. 1 and 2 inform that the imposed laccase-catalysis were adequate toward attaining oxidative species of FA and EGCG that can bind to curdlan. Differential binding stemming from different preparative sequences might merely reflect the relative difference in species composition over incubation time.

3.2. Effect of phenolic compounds (native, oxidised) on the G' of heat-set emulsion gels and their aqueous phase

Fig. 3 shows the G' of curdlan (at 10% w/w) and CFN together in aqueous medium at 21 °C after cooling from 95 °C, with or without dosing of 1 mM FA. With such heat-cool cycle, un-annealed, heat-mobilised strands of curdlan would form tight triplex helices (Xiao et al., 2017) that network to confer bulk structural strength. Before heating, the fibre samples were prepared according to sequence A/B with or without laccase. With laccase but not FA, the fibres displayed a G' value of 12616 Pa, higher than in fibres alone and fibres with native FA. Our preliminary Folin Ciocalteu assay on CFN revealed that, at the dosage used in the aqueous phase in our emulsion gel system, CFN contributed an average of 0.6 mM gallic acid equivalent in total phenolic content. Inherent phenolic content in CFN had also been reported before in literature (Gedikoğlu et al., 2021). Thus, such high G' could be attributed to fibre crosslinking supported by laccase-catalysed covalent binding between the inherent phenolic content in CFN and the trace nitrogen in the fibres. The highest G' at 14067 Pa, however, was attained with extraneous phenolic content that was laccase-oxidised (see arrow). This attests to the relevance of studying the effect of extraneous phenolic compounds on the G' of heat-set emulsion gels and their aqueous phase, as depicted in Fig. 4.

To elaborate, Fig. 4 shows at 20 °C after one heat-cool cycle the influence of oxidised and native phenolic compounds on the G' of heat-set emulsion gel hybrids (Figs 4A and 4C) and their corresponding aqueous phase (Figs 4B and 4D). The influence of the structuring process sequence was highlighted in Figs 4A and 4B using FA as an example, whereas Figs 4C and 4D reveal the impact of FA, EGCG, and OPC in the samples, prepared according to sequence C for comparison purpose. First, as evident in Fig. 4A, sequences C and D led to higher G' in the magnitudes of ≥ 6000 Pa, especially for sequence D. If considering only the effect of oxidised FA, the order of sequences leading to decreasing G' in the emulsion gel hybrids was D (yielding 7187 Pa) > C > B > A (yielding 3905 Pa) (see long arrow). This trend was opposite to the higher reactivity between laccase-oxidised FA and curdlan observed with sequence A/B than with sequence C/D in Fig. 2A. Considering the viscous presence of CFN in the samples in Fig. 4, we hold the viewpoint that kinetic influence could outweigh thermodynamic influence, such that the decreasing order of effectiveness from sequences D, C, to B was inversely correlated to the viscosity of their respective aqueous phase during oil emulsification (i.e., CFN slurry; curdlan + CFN slurry; and potentially crosslinked curdlan + CFN slurry). The less viscous the aqueous phase or bulk viscosity during emulsification, the potentially higher convective migration of emulsifying fibre strands to freshly cleaved oil-water interface to stabilise oil droplets, and in turn the smaller the oil droplets could be in an emulsion gel to enhance G' . Moreover, with sequence B there was higher risk of bridging flocculation by higher-weight polymeric species during the shear-induced collisions of emulsification (Tan & Phoon, 2023). On the other hand, the weakest outcome was seen with sequence A, whereby oil droplets were already formed before laccase was dosed followed by the

16-h enzymic reaction. As indicated by the broken arrow in Fig. 2A, such arrangement in the sequence was less favorable, plausibly due to excessive clustering of inter-oil droplets leading to micro-phase separation. Otherwise, the advantage of incorporating oxidised FA to promote crosslinking was clear, as noted in the cases with sequences B, C, and D.

This brings up the point of validating where the crosslinking sites which boosted bulk G' were, as in whether it was the aqueous phase or the interfaces of oil-droplets which had more domineering effect. For sequences A and B, there was prolonged reaction time between trace nitrogen in the dispersed fibres and oxidised FA products progressively generated over the 16 h of enzymic reaction. The reaction could lead to considerable increase in viscosity in the FA-grated polymer (Aljawish et al., 2014) and an increase in G' (Fig. 4B). This increase in G' was 1752 Pa in the curdlan-only slurry (see left broken arrow in Fig. 4B), while the increase in G' in the curdlan + CFN aqueous phase was 2641 Pa and only modestly higher (see right broken arrow in Fig. 4B). Thus, it is deducible that much of the structural strength in the aqueous phase arose from curdlan. On the other hand, for sequences C and D, the time between the dispersion of fibres and the actual rheological measurement was relatively less (at least 16 h shorter). G' of the curdlan + CFN slurry was higher than that for sequences A and B (17443 Pa vs. 11426 Pa, see Fig. 4B), implying less time for activated CFN strands to reaggregate hydrophobically. However, both native and oxidised FA seemed to promote this hydrophobic aggregation in CFN to cause lower G' values in the aqueous phase (17443 Pa vs. 13811-13922 Pa, see solid arrow in Fig. 4B). This was in opposite trend to what was discussed earlier on how oxidised FA caused stronger corresponding emulsion gel hybrids. The only way to explain this was that crosslinking at inter-oil-droplet bridges offset the adverse effect of hydrophobic aggregation on G' . From our perspective, at high oil load of 50% (w/w), crosslinking at inter-oil-droplet bridges as induced by oxidised FA-grafting of interfacial curdlan encouraged the active-filler phenomenon (Oliver et al., 2015), which was more pivotal than crosslinking in the aqueous phase in boosting bulk G' . In section 3.5, the mechanism of interfacial crosslinking would be further discussed.

Figs 4C and 4D include the G' analysis with native and oxidised EGCG and OPC, showing repeats in the general trends observed with FA in Figs 4A and 4B at the same aqueous concentration of 1 mM. However, unlike FA, both EGCG and OPC caused noticeable increase in the emulsion gel hybrid G' even without laccase-catalysed oxidation (see arrows in Fig. 2C). It could be that with their higher MWs than FA, there was higher density of hydroxyl groups to form hydrogen bonds with curdlan, promoting network formation in turn. In the case with OPC, it might be because it was already oxidised when supplied (see Section 3.1), such that there was lack of further significant increase in the emulsion gel hybrid G' with enzyme-treated OPC. Its emulsion gel hybrid G' stayed within 6224-6401 Pa, similar as that of oxidised FA at 6446 Pa despite the higher mass used during sample preparation (due to its

higher MW, circa 3 times of FA). In this regard, the OPC ingredient was less interesting in functional potential. On the other hand, EGCG was interesting in its own way. Based on Fig. 4D, the aqueous phase with native and oxidised EGCG seemed to have less CFN reaggregation, judging from the lower decline in G' to 15633-15899 Pa (see black arrow), compared to the cases with OPC (to 13829-14368 Pa) and FA (13811-13922 Pa) (see broken arrows). Native EGCG has a carbonyl group which reduces its hydrophobicity. Thus, our conjecture is that any hydrophobic interaction with CFN was relatively weaker for EGCG than with OPC and FA, native or oxidised. With oxidised EGCG-based emulsion gel hybrid, its low extent of CFN reaggregation in the aqueous phase was congruent with its outstanding G' of 7735 Pa.

To consolidate the results from this first comparison of G' values, the phenolic compound MW was not the most critical factor in influencing the G' of emulsion gel hybrids. With suitable sequencing of steps in sample preparation, high G' in emulsion gel hybrids could be attained. Oxidised EGCG was promising as a network crosslinker in terms of conferring its emulsion gel hybrid the highest G' . FA stayed interesting for more investigation due to the lack of dark colour development during oxidation, which is important for product acceptance (Liu et al., 2014). Both EGCG and FA would be discussed further in terms of their influence on the thermal and textural properties of emulsion gel hybrids (see Section 3.4).

3.3. Correlation between G' in emulsion gel samples and oil aggregate size

Using the FA-based emulsion gel hybrid system as an example, we sought to understand how the extent of inter-oil-droplet crosslinking might impact the bulk G' . For which, we adjusted the formulation via incremental approaches, measured the oil aggregate sizes of the emulsion gel hybrid templates seen on their micrographs, and did rheological analysis after one heat-cool cycle. Sequences C and D were followed in the sample preparation. The concentration of FA in the aqueous phase was increased from 1 mM to 4 mM. Additionally, 0.2% to 0.4% (w/w) of polyglutamic acid (PGA) was deliberately incorporated into the formulation to increase the trace protein content, facilitating crosslinking with oxidised FA. Besides, PGA-phenolic acid interaction through hydrogen bonds (Wang et al., 2019) among carboxylic, hydroxyl, and amine groups might contribute to enhancing the active filler effect.

Figs 5A and 5B depict the outcome of the refined formulations. In Fig. 5A, the solid arrows indicate rising G' in the heat-set emulsion gel system with increasing concentrations of FA (native or oxidised) and PGA, leading to an optimum value. Correspondingly, the average oil aggregate size in the microstructure decreased to a minimum, as supported by the micrographs in Fig. 5B and the measurements in Table 1. For sequences C and D, the respective optimum G' of 6873 Pa and 8104 Pa

were found in samples prepared with 4mM FA (native or oxidised) and 0.2% (w/w) PGA, with the corresponding minimum average oil aggregate size within 21-23 μm and its size distribution narrow. Conversely, with further addition of FA (native or oxidised) and PGA, G' declined as denoted by the broken arrows in Fig. 5A. There was also a parallel increase in the average oil aggregate size.

The positive trend denoted by the solid arrows in Fig. 5A might be correlated to the control of oil droplet coalescence. To elaborate, immediately after oil droplet breakup during emulsification, their coalescence would depend on the stiffness in the formed polymeric interfacial layer and/or their external proximity. Such stiffness could be enhanced by effective adsorption of mainly CFN onto the oil-water interface, as well as crosslinking at inter-oil-droplet bridges. This helped to keep the oil droplet and oil aggregate sizes small to contribute to high G' (Tan & Phoon, 2023). Then, as the combined concentrations of FA (native, oxidised) and PGA got higher, it could be that further inter-oil-droplet crosslinking was stimulated such that the small aggregate size advantage to G' was increasingly lost. Another possibility behind the decline in G' was the coalescence of oil aggregates (Fredrick et al., 2010; Xu et al., 2022) due to weakened inter-oil-droplet bridges. The stiffness at the bridges might be compromised by electrostatic repulsion among the fibres, resulting from the excessive surface negative charge of the PGA bound to them (Hu et al., 2020). To summarise, there seemed to be an optimal microstructure in the emulsion gel hybrid template system to yield high G' following heat-setting. For which, keeping the average oil aggregate size small would be beneficial.

3.4. Thermal properties of emulsion gel hybrids with phenolic compounds

Emulsion gel hybrid systems bearing 1 mM EGCG or FA in aqueous phase were further analysed to evaluate thermal behaviour more closely. The temperature dependence of viscoelastic moduli was tracked over two consecutive heat-cool cycles within 20-100 $^{\circ}\text{C}$, with rapid heating to 70 $^{\circ}\text{C}$ in the first cycle and slower heating to 100 $^{\circ}\text{C}$ in the next (Fig. 6). From this test design, we aimed to detect what structural transition(s) the samples underwent and how they developed thermo-irreversibility. First, we did not find any $G'-G''$ crossover which would represent sol-gel/gel-sol transition (Figs 6A and 6B). This is conceivably because despite the high moisture content in the aqueous phase (> 85% w/w), under the high aqueous curdlan concentration of 10% (w/w), mobility of water molecules surrounding the curdlan helices in the swollen particulates was impeded (Zhang et al., 2002). The ascertaining of thermo-irreversibility was complicated by curdlan concentration, rate of temperature change, and how the changeover to irreversibility may not necessarily occur at a singular temperature with absoluteness (Funami et al., 1999; Hirashima et al., 1997). Nonetheless, in Figs 6A, 6C, and 6D, G' was on the rise from 70 $^{\circ}\text{C}$ in all the second heating ramps, reflecting the renaturing of residual, un-annealed, and heat-mobilised strands (Xiao et al., 2017) into triplex helices which then aggregated to form

networking microfibrils (Harada et al., 1991). This infers that gel-setting during the first heat-cool cycle was not yet as dominantly thermo-irreversible as the second heat-cool cycle. Yet, there was this same recurring, remarkable climb in G' at $\sim 40^\circ\text{C}$ during cooling. Zhang et al. (2002) ascribed this climb in G' to single helical transition of un-annealed (non-triplex) strands, accompanying which was association among the single helices and triplexes via hydrogen bonding (Harada et al., 1991).

This then begs the next question of whether EGCG and FA (native, oxidised) might influence the inter-triplex packing differently. The hysteresis in the transition loops of the second heat-cool cycle (portrayed by the shaded area in Fig. 6A) is plausibly associated with inter-triplex movement, depending on the interspersions, stiffening, and relaxing of un-annealed portions (Zhang et al., 2002). A smaller hysteresis area could mean less un-annealed portions to relax in the second heating ramp and/or restructure in the second cooling ramp. Based on Table 2, which compiles the calculated average hysteresis area from Figs 6A, 6C, and 6D, the hysteresis area of curdlan gel and curdlan + CFN gel samples was similar (29794 versus 29802 $\text{Pa}\cdot^\circ\text{C}$). This suggests the non-interference of CFN on curdlan triplex network formation in the emulsion gel hybrid system. Of practical significance is that oxidised FA, which yielded the lowest average hysteresis area of 12054 $\text{Pa}\cdot^\circ\text{C}$, showed potential of strengthening the inner structure of the curdlan microfibril. Congruent to this indication were the T_g results of freeze-dried curdlan powders in Table 3. In the first batch of samples, curdlan was priorly hydrated with and without FA at room temperature, whereby the native curdlan helices were still closely packed (Xiao et al., 2017). Thus, although this led to increase in bound water and lower T_g values ($258.15\text{--}258.25^\circ\text{C}$) compared to literature reported values ($\sim 280^\circ\text{C}$) (Suzuki et al., 2022), the moisture content after freeze-drying was still controlled low at 0.98% (w/w) as determined by oven-drying method. Conversely, in the second batch of samples, curdlan was priorly hydrated with and without FA at 70°C , whereby the spatial gaps among the helices were wider (Xiao et al., 2017). This encouraged uptake of FA into this space along with more water imbibition, higher bound moisture after freeze-drying (1.73% w/w), and even lower T_g values ($246.50\text{--}248.65^\circ\text{C}$). While the risk of uneven moisture distribution in freeze-dried samples might be compounded by the minute analyte amounts in DSC measurement (within 3.4–6.8 mg), the higher T_g values in emulsion gel hybrids with FA ($247.95\text{--}248.65^\circ\text{C}$) than that without (246.50°C) are still of practical significance.

3.5. Interpretation of strengthening of emulsion gel hybrids by ferulic acid

As discussed in Section 3.4, FA enabled the strengthening of curdlan inter-triplex network in the emulsion gel hybrid system. A likely further enhancing mechanism was crosslinking of fibres at the oil-water interface for stronger active-filler effect, as facilitated by covalent binding between trace nitrogen in the fibres and quinones from laccase-oxidised FA. To validate this second mechanism,

interfacial fibre was separated from FA-containing emulsion gel hybrid templates and detected for quinones by the NBT-glycinate redox staining assay. MCT was specially used for the oil load in place of sunflower oil in creating the emulsion gel hybrid templates. This was in consideration of the presence of phenolic compound in sunflower oil in the form of chlorogenic acid (Náthia-Neves & Alonso, 2021), which could cause false positive result in the NBT-glycinate assay. Incidentally, the shorter fatty acid chain length in MCT meant a weaker emulsion gel hybrid template at the same oil percentage, and thus easier breakdown of the template by centrifugal means for interfacial fibre recovery. Fig. 7 reveals that in the retrieved interfacial fibre, besides the expected presence of CFN since it was used for its emulsifying functionality, curdlan was also present. Fig. 8 confirms that the NBT-glycinate assay has higher sensitivity to laccase-oxidised FA (quinones) over the native phenolic acid, and is thus suitable for assessing retrieved interfacial fibre for quinones. For which, to improve data reliability, the interfacial fibre was washed twice with distilled water to remove residual FA from the aliquot retrieval process. Table 4 confirms adequate washing as seen in the negligible absorbance values of reduced NBT. Most importantly, at the same dosage of FA, Table 4 shows higher average absorbance value in the interfacial fibre with laccase-catalysed oxidation than that without (1.08 Abs versus 0.47 Abs), signifying the generation of quinones with the use of laccase.

The FA-enabled strengthening of curdlan inter-triplex network and crosslinking in interfacial fibre manifested into mechanically stronger heat-set emulsion gel structures under high deformation, as according to the results of compressive stress-strain profiling at room temperature (Fig. 9, Table 5). With 1 mM FA (native or oxidised) in the aqueous phase, hardness increased from 928 g to 1341-1558 g, toughness grew from 530,023 to 742,642-842,265 Pa.%, and the change in stiffness across 60-70% strain rose from 60,500 to 88,400-103,100 Pa. In particular, the results with oxidised FA was outstanding ($p < 0.05$). Comparatively, the emulsion gel hybrids comprising native or oxidised EGCG did not exhibit the same mechanical strength as those with native or oxidised FA under high deformation strain. From m/z data observed during LC-MS analysis (data not shown), there was generally higher MW species seen in oxidised EGCG than in oxidised FA. The higher average hysteresis areas and weaker textural properties of EGCG (native, oxidised) might be due to steric hindrance on the inter-triplex packing of curdlan. To sum up, the use of oxidised FA in an emulsion gel system can be useful for structuring oil into a temperature- and high-strain stable semi-solid form.

4. Conclusion

This experimental study examines how an emulsion gel hybrid system structured by commercially mass-produced curdlan and citrus fibre could be mechanically strengthened at relevant food serving

temperatures, using oxidised phenolic compounds as catalysed by laccase. Three phenolic compounds were studied, namely epigallocatechin-3-gallate, ferulic acid, and grape seed extract derived ortho-proanthocyanidin. Of which, oxidised ferulic acid yielded the most notable achievement at very low concentrations (in mM), under suitable sequencing of steps which kept the aqueous phase viscosity low during emulsification. Underlying the improvement in elastic modulus was a microstructure characterised by interlinked oil droplets in well-controlled aggregate sizes, facilitated by enhanced curdlan inter-triplex network and covalent binding between trace nitrogen in the fibres and quinones from laccase activity. Meanwhile, a molecularly larger phenolic compound need not necessarily be a more effective crosslinking agent. There has been increasing research efforts on expeditious production of ferulic acid from natural sources (Effendi & Ng, 2023; Ferri et al., 2020; Sibhatu et al., 2021), rising popularity of laccase applicability in food processing (Backes et al., 2021; Chai et al., 2020; Li et al., 2020), and the facile alternative to laccase-catalysed oxidation of phenolic compounds by oxygen bubbling at alkaline pH (Zhang et al., 2023). As such, our approach provides reference value for natural and economically pragmatic creation of stiff animal fat mimetics out of unsaturated edible oils. Our next research step would focus on combining this approach with another mechanism in aqueous phase fibre network strengthening, toward further enhanced functional prototyping in this direction.

Declaration of interest

None.

Author statement

Zhan Lun Alan Tan: Conceptualisation, Methodology, Investigation, Formal analysis, Validation, Visualisation, Writing - original draft. Amanda Xin Yi Sng: Methodology, Investigation, Formal analysis, Validation, Visualisation, Writing – review and editing. Choy Eng Nge: Methodology, Formal analysis, Validation, Visualisation, Writing – review and editing. Pui Yeu Phoon: Funding acquisition, Conceptualisation, Supervision, Methodology, Investigation, Formal analysis, Validation, Visualisation, Writing – original draft and editing.

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Table 1. Oil aggregate size measurements of emulsion gel samples prepared according to sequences C and D, with and without ferulic acid (native, laccase-oxidised) and polyglutamic acid.

Sample	Sequence C			Sequence D		
	Size (μm) $\pm$ SD			Size (μm) $\pm$ SD		
F + oil	47	$\pm$	30	43	$\pm$	11
1 mM FA + F + oil	41	$\pm$	16	34	$\pm$	6
1 mM FA* + F + oil	26	$\pm$	6	25	$\pm$	2
1 mM FA + F + 0.2% (w/w) PGA + oil	30	$\pm$	6	34	$\pm$	8
1 mM FA* + F + 0.2% PGA + oil	26	$\pm$	8	23	$\pm$	3
4 mM FA + F + 0.2% PGA + oil	23	$\pm$	6	28	$\pm$	2
4 mM FA* + F + 0.2% PGA + oil	35	$\pm$	15	21	$\pm$	2
4 mM FA + F + 0.4% PGA + oil	27	$\pm$	6	32	$\pm$	5
4 mM FA* + F + 0.4% PGA + oil	34	$\pm$	15	25	$\pm$	8

Notes.

Abbreviations: F - citrus fibre and curdlan; FA - ferulic acid; PGA - polyglutamic acid; SD - standard deviation.

*Laccase-oxidised.

Table 2. Average hysteresis area of select samples, determined from 20-60 °C during the second heat-cool cycle of rheological measurement.

Sample	Average hysteresis area $\pm$ SD (Pa. °C)		
CG	29794	$\pm$ 1127	^a
CG + CFN	29802	$\pm$ 627	^a
F + oil	20071	$\pm$ 3334	^a
1mM FA + F + oil	14640	$\pm$ 3222	^{ab}
1mM FA* + F + oil	12054	$\pm$ 3055	^b
F + oil	20071	$\pm$ 3334	^a
1mM EGCG + F + oil	13304	$\pm$ 6455	^a
1mM EGCG* + F + oil	16769	$\pm$ 542	^a

Notes.

The aqueous concentrations of curdlan and phenolic compounds were standardised to 10% (w/w) and 1 mM, respectively.

All emulsion gel samples were prepared according to sequence D and had 50% (w/w) sunflower oil load.

The results were analysed by one-way ANOVA with Tukey post-hoc test ($p < 0.20$).

Abbreviations: CFN - citrus fibre; CG - curdlan; EGCG - epigallocatechin-3-gallate; F - citrus fibre and curdlan; FA - ferulic acid; SD - standard deviation.

*Laccase-oxidised.

Table 3. Onset glass transition temperature (T_g) readings of freeze-dried samples prepared from 10% (w/w) curdlan suspensions with and without 1 mM ferulic acid (native, laccase-oxidised).

Batch	Sample	Onset T _g ± SD (°C)	Moisture content ± SD (% w/w)
1	CG	258.15 ± 0.78 ^a	0.98 ± 0.40
	FA*-CG	258.25 ± 0.21 ^a	
2	CG	246.00 ± 1.40 ^a	1.73 ± 0.12
	FA-CG	248.65 ± 0.07 ^b	
	FA*-CG	247.95 ± 0.35 ^{ab}	

Notes.

For batch 1, the suspensions were not subjected to heating above room temperature. For batch, the suspensions were incubated at 70 °C for 10 min in their thermal history.

The results were analysed by one-way ANOVA with Tukey post-hoc test ($p < 0.10$).

Abbreviations: CG - curdlan; FA - ferulic acid; SD - standard deviation.

*Laccase-oxidised.

Table 4. Average absorbance readings for assessment of nitro blue tetrazolium (NBT)-glycinate redox staining assay. All samples were added with 0.41 mM NBT in 2 M potassium glycinate at 1:4 mass ratio, and read at 560 nm in 150 uL volumes in a 96-well clear plate with flat bottom. The samples included aqueous-washed, re-dispersed interfacial fibre separated from centrifuged emulsion gel templates, prepared using 1 mM of either native or laccase-oxidised ferulic acid. Also analysed was solvent from the preceding aqueous washings.

Sample	Average absorbance $\pm$ SD		
A: Interfacial fibre from emulsion gel with native FA	0.47	$\pm$	0.01 ^b
B: Interfacial fibre from emulsion gel with laccase-oxidised FA	1.08	$\pm$	0.02 ^a
Solvent from 1 st preceding washing of A	0.09	$\pm$	0.00 ^d
Solvent from 1 st preceding washing of B	0.14	$\pm$	0.01 ^c
Solvent from 2 nd preceding washing of A	0.04	$\pm$	0.00 ^e
Solvent from 2 nd preceding washing of B	0.06	$\pm$	0.00 ^{de}
Negative control: 0.41 mM NBT in 2 M potassium glycinate	0.02	$\pm$	0.00 ^e

Notes.

The emulsion gel template samples were prepared according to sequence D and had 40% (w/w) caprylic triglyceride oil load.

The results were analysed by one-way ANOVA with Tukey post-hoc test ($p < 0.05$).

Abbreviation: FA - ferulic acid; SD - standard deviation.

Table 5. Average values of toughness, hardness, and change in stiffness derived from stress-compressive strain curves of select heat-set emulsion gels, prepared according to sequence D, with and without phenolic compound (native, laccase-oxidised).

Phenolic compound content in aqueous phase of emulsion gel sample	Toughness $\pm$ SD (Pa.%) within 0-75% (Pa.%)	Change in stiffness $\pm$ SD (Pa) within 60-70% strain	Hardness $\pm$ SD (g) at 75% strain
None	530,023 $\pm$ 89,342 ^a	60,500 $\pm$ 9,500 ^a	928 $\pm$ 140 ^a
1 mM FA	742,642 $\pm$ 12,589 ^b	88,400 $\pm$ 4,300 ^c	1,341 $\pm$ 34 ^b
1 mM FA*	842,265 $\pm$ 35,136 ^c	103,100 $\pm$ 5,700 ^d	1,558 $\pm$ 94 ^c
1 mM EGCG	718,465 $\pm$ 8,523 ^b	73,300 $\pm$ 2,700 ^b	1,200 $\pm$ 30 ^b
1 mM EGCG*	703,477 $\pm$ 42,140 ^b	82,100 $\pm$ 2,500 ^{bc}	1,278 $\pm$ 49 ^b

Notes.

All emulsion gel samples had 50% (w/w) sunflower oil load and 10% (w/w) curdlan in aqueous phase.

The results were analysed by one-way ANOVA with Tukey post-hoc test ($p < 0.05$).

Abbreviations: EGCG - epigallocatechin-3-gallate; FA - ferulic acid; SD - standard deviation.

*Laccase-oxidised.

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Figure captions

Fig. 1. Liquid Chromatography-Mass Spectrometry (LC-MS) base peak chromatogram (BPC) results for aqueous solutions of (A) native ferulic acid (FA); (B) 4 mM FA oxidised with 0.34% (w/w) laccase for 16 h; (C) native epigallocatechin-3-gallate (EGCG); (D) 1 mM EGCG oxidised with 0.34% (w/w) laccase for 16 h; (E) ortho-proanthocyanidin (OPC) from grape seed extract; (F) 1 mM OPC oxidised with 0.34% (w/w) laccase for 16 h; and (G) laccase.

Fig. 2. Solid-state CP/MAS ^{13}C -NMR spectra of curdlan freeze-dried from various 10% (w/w) aqueous suspensions mixed with 0.34% (w/w) laccase and 1 mM of either (A) ferulic acid or (B) epigallocatechin-3-gallate. In the bottom spectra (i), the phenolic compound was oxidised by laccase in the presence of curdlan at 45 °C for 16 h, as per sequence A/B. In the top spectra (ii), the phenolic compound was pre-oxidised by laccase at 45 °C for 16 h before curdlan was mixed in, as per sequence C/D.

Fig. 3. Thermometer chart showing average storage modulus (G') of heat-set fibre samples at 21 °C. The samples were prepared according to sequence A/B, held at 16 h with or without laccase, and with or without dosing of 1 mM ferulic acid. The G' measurements were from oscillatory rheology at 1 Hz, 0.2% strain, following a heat ramp from 20 °C to 95 °C at 38 °C/min and immediate cooling back to 20 °C at 11 °C/min. Abbreviations: F - citrus fibre and curdlan; FA - ferulic acid. *Presence of laccase.

Fig. 4. Thermometer chart of average storage modulus (G') of heat-set emulsion gel samples and their corresponding aqueous phases, with and without phenolic compounds (native, oxidised). (A) Emulsion gel samples prepared according to different sequences A, B, C, and D, with ferulic acid as the phenolic compound. (B) Curdlan suspensions and slurries comprising curdlan and citrus fibre (i.e., aqueous phase) prepared according to sequence A/B and sequence C/D, with ferulic acid as the phenolic compound. (C) Emulsion gel samples with different phenolic compounds prepared according to sequence C. (D) Corresponding aqueous phase samples of the emulsion gels in (C), prepared according to sequence C. G' measurements were from oscillatory rheology at 1 Hz, 0.2% strain, following a heat ramp from 20 °C to 95 °C at 38 °C/min and immediate cooling back to 20 °C at 11 °C/min. The aqueous concentrations of curdlan and all phenolic compounds were standardised to 10% (w/w) and 1 mM, respectively, and all emulsion gel samples had 50% (w/w) sunflower oil load. Abbreviations: CG - curdlan; EGCG - epigallocatechin-3-gallate; F - citrus fibre and curdlan; FA - ferulic acid; OPC - proanthocyanidin. *Laccase-oxidised.

Fig. 5. (A) Average storage modulus (G') of heat-set emulsion gel samples prepared according to sequences C and D, with and without ferulic acid (native, laccase-oxidised) and polyglutamic acid. G' measurements were from oscillatory rheology at 1 Hz, 0.2% strain, following a heat ramp 20 °C to 95 °C at 38 °C/min and immediate cooling back to 20 °C at 11 °C/min. All emulsion gel samples had 50% (w/w) sunflower oil load and 10% (w/w) curdlan in aqueous phase. (B) Corresponding epifluorescence microscopy images of the emulsion gel samples in (A), grouped into clusters with different, colour-aligned borders. Abbreviations: F - citrus fibre and curdlan; FA - ferulic acid; PGA - polyglutamic acid. *With laccase-oxidised ferulic acid. ✓ With smallest oil droplets/aggregates of narrow size distribution. *Laccase-oxidised. Scale bar: 100 μm .

Fig. 6. Tracking of viscoelastic moduli of select samples in two consecutive heat-cool cycles. (A) Average storage modulus (G') of 10% (w/w) curdlan suspension and a slurry comprising 10% (w/w) curdlan and citrus fibre. (B) Average loss modulus (G'') of the same samples in (A). (C) G' of emulsion gel samples with and without ferulic acid (native, laccase-oxidised), prepared according to sequence D. (D) G' of emulsion gel samples with and without epigallocatechin-3-gallate (native, laccase-

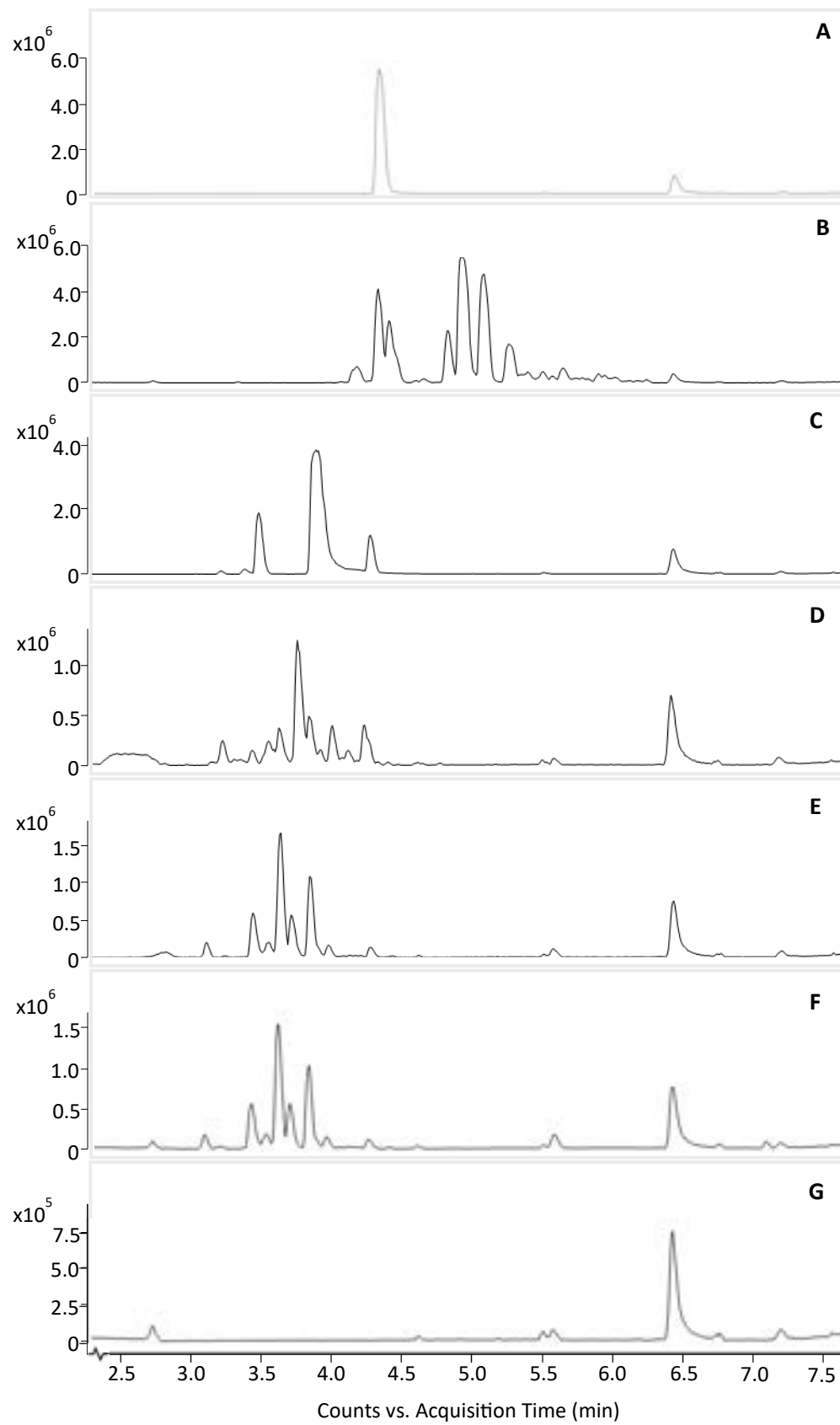
oxidised), prepared according to sequence D. G' measurements were from oscillatory rheology at 1 Hz, 0.2% strain. The first heat-cool cycle had a heat ramp from 20 °C to 70 °C at 25°C/min, holding for 1 min, followed by cooling from 70 °C to 20 °C at 10°C/min and subsequent holding for 1 min. The second heat-cool cycle had a heat ramp from 20 °C to 100 °C at 10 °C/min, holding for 1 min, and cooling from 100 °C to 20 °C at 10°C/min. All emulsion gel samples had 50% (w/w) sunflower oil load and 10% (w/w) curdlan in aqueous phase. Abbreviations: CFN - citrus fibre; CG - curdlan; EGCG - epigallocatechin-3-gallate; F - citrus fibre and curdlan; FA - ferulic acid. *Laccase-oxidised.

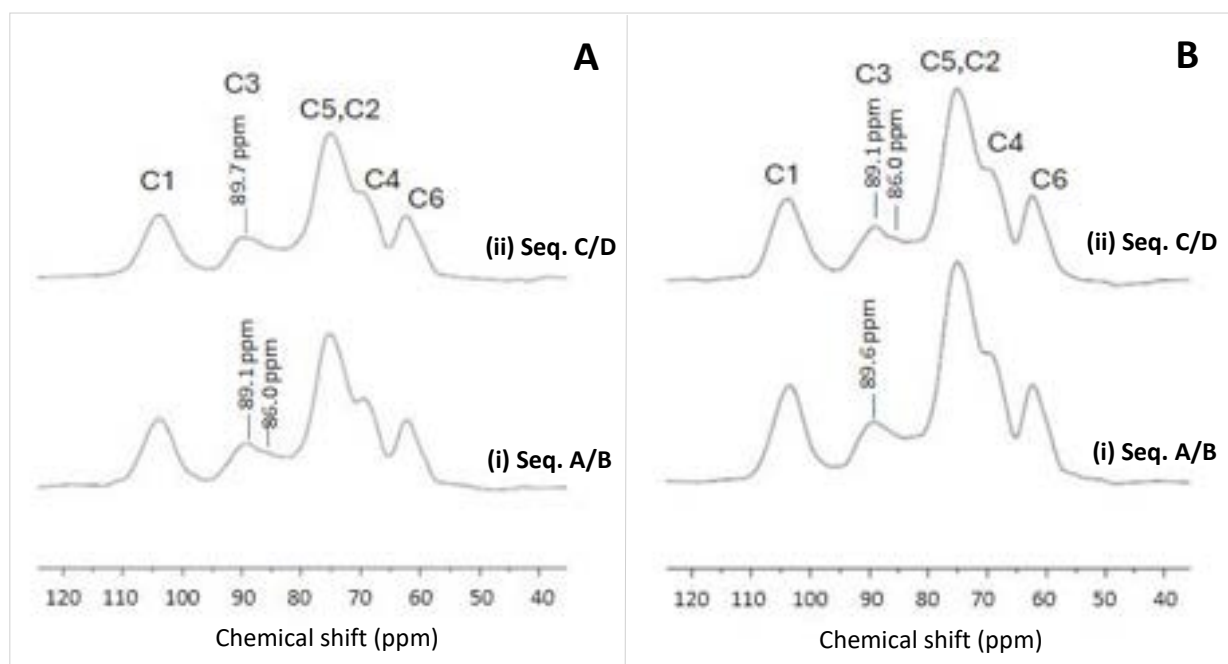
Fig. 7. Interfacial fibre (A) congregated between an upper oil layer and a bottom layer of emulsion gel template following centrifugation (see asterisk), and (B) its merged epi-fluorescence micrographs showing the co-presence of curdlan and citrus fibre. The micrographs were derived from an emulsion gel template prepared with 1 mM of laccase-oxidised ferulic acid. Curdlan was stained blue by sodium 8-anilino-1-naphthalenesulfonate, and citrus fibre was stained red by Direct Red 23 dye. Scale bar: 100 μ m. Magnification: 20 $\times$.

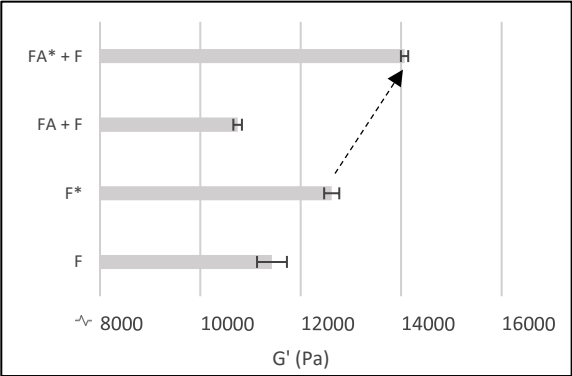
Fig. 8. Photo montage of samples from freeze-dried curdlan assessed for nitro blue tetrazolium (NBT) reduction activity after 5, 45, and 90 minutes. The samples comprised of (1) curdlan; (2) curdlan with native ferulic acid; (3) curdlan with laccase-oxidised ferulic acid prepared according to sequence A/B; and (4) curdlan with laccase-oxidised ferulic acid prepared according to sequence C/D. In row A, the samples were added with 0.41 mM NBT in 2 M potassium glycinate (pH 11) at 1:10 mass ratio. Row B shows corresponding controls whereby only 2 M potassium glycinate (pH 11) was added. All images were taken at the same light condition and camera settings.

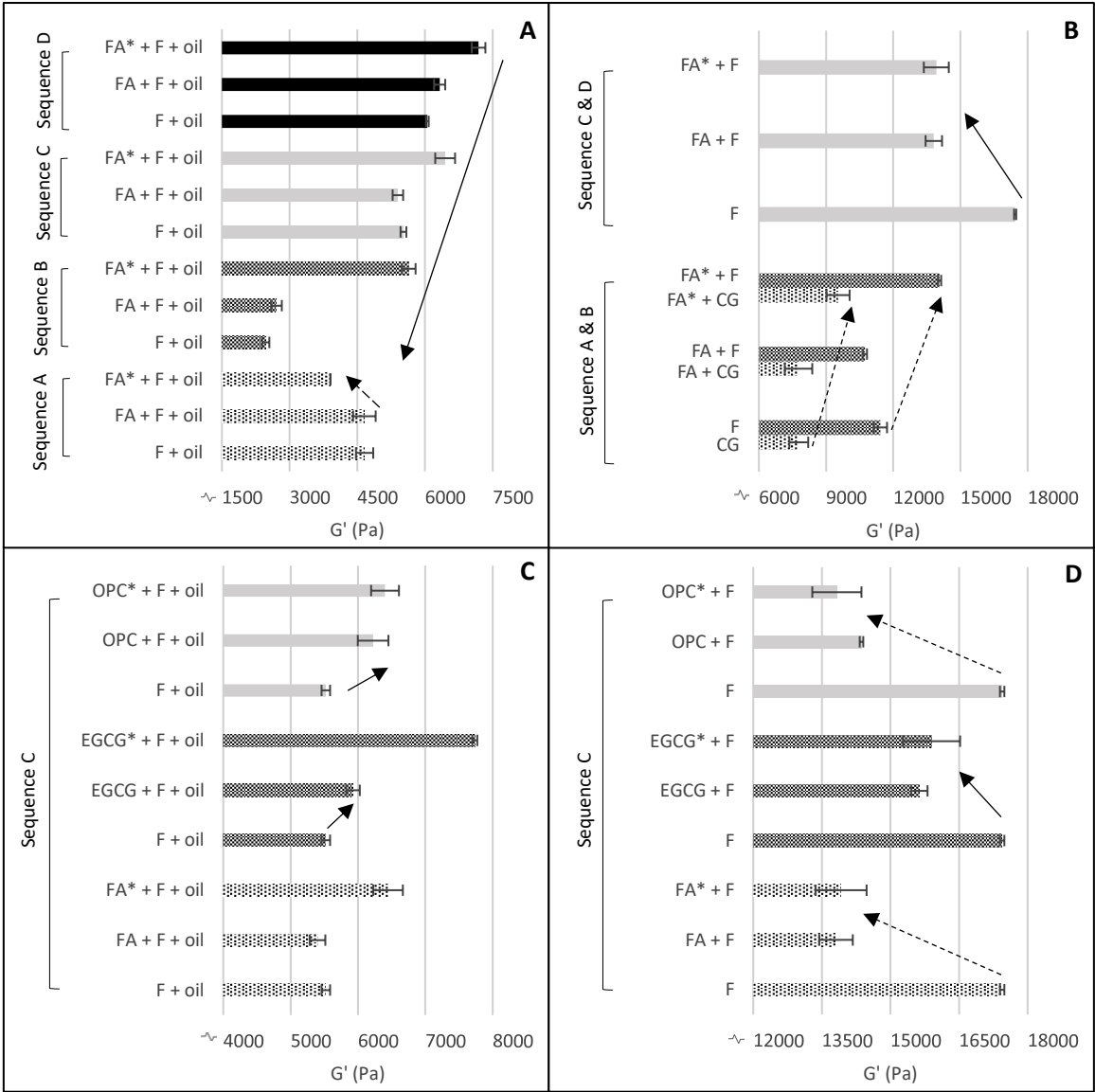
Fig. 9. Average stress-compressive strain curves of heat-set emulsion gel hybrids prepared according to sequence D, with and without (A) ferulic acid and (B) epigallocatechin-3-gallate. The test speed was set at 1.0 mm/s. All emulsion gel samples had 50% (w/w) sunflower oil load and 10% (w/w) curdlan in aqueous phase. Abbreviations: EGCG - epigallocatechin-3-gallate; F - citrus fibre and curdlan; FA - ferulic acid. *Laccase-oxidised.

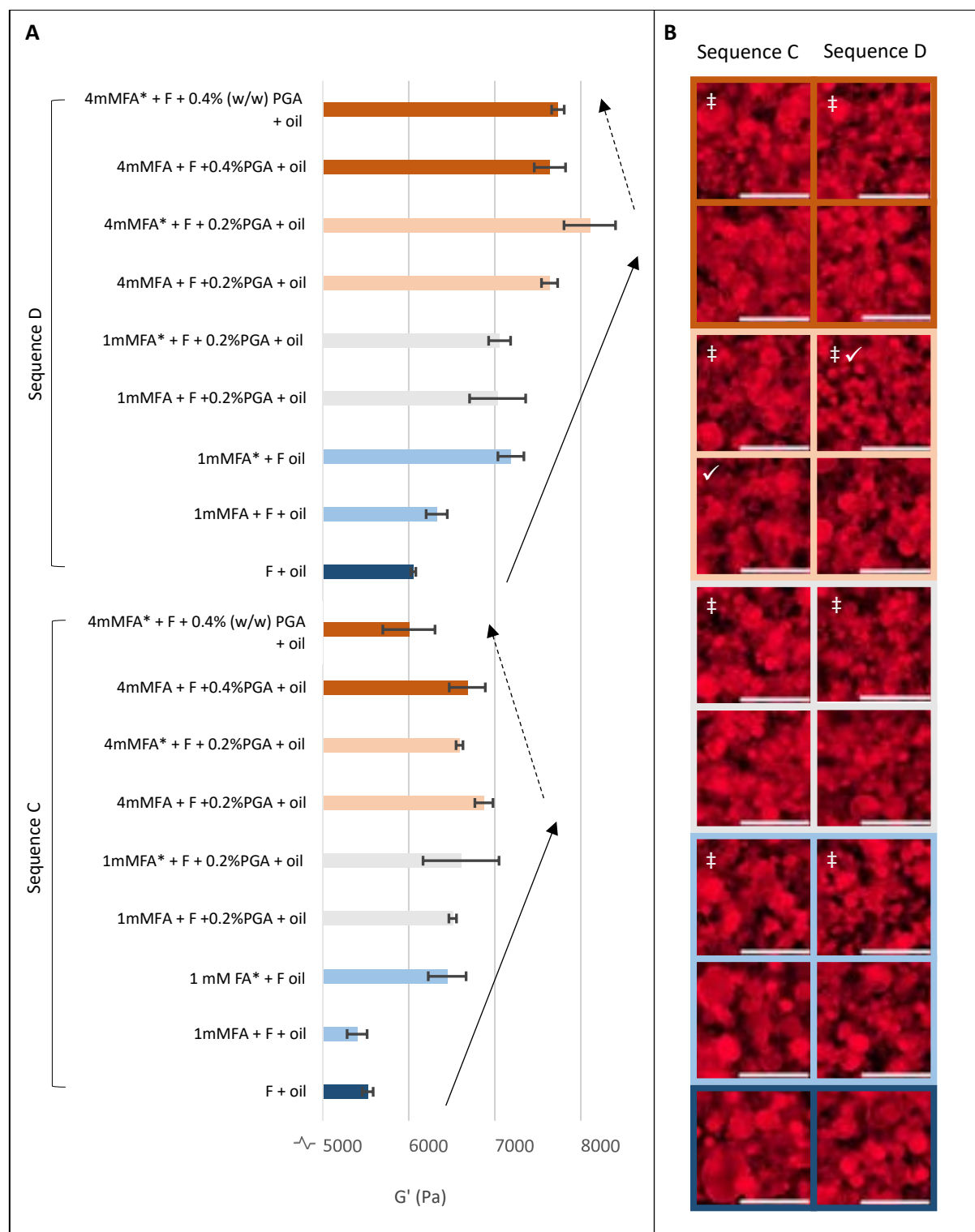
Figure 1

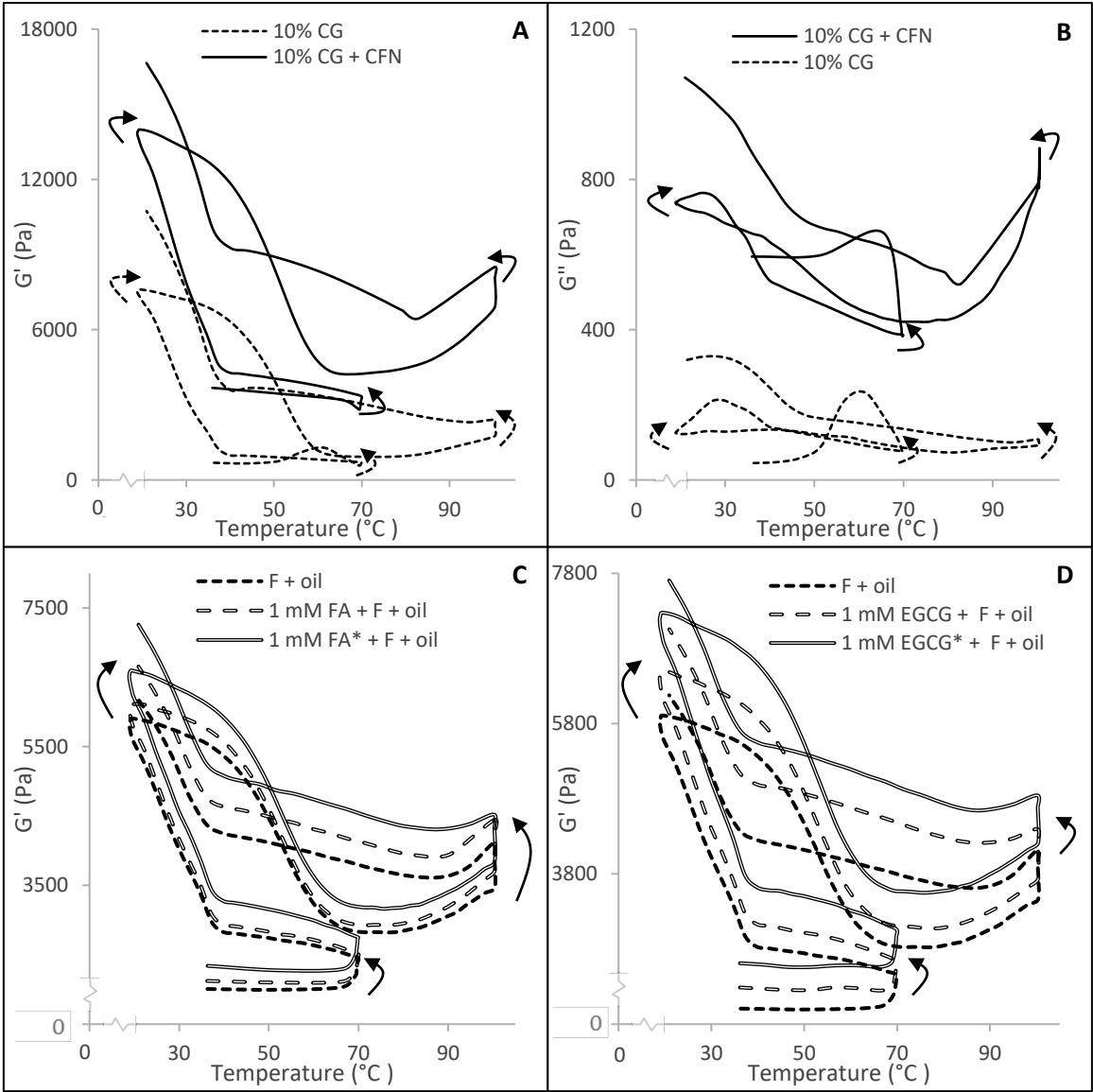












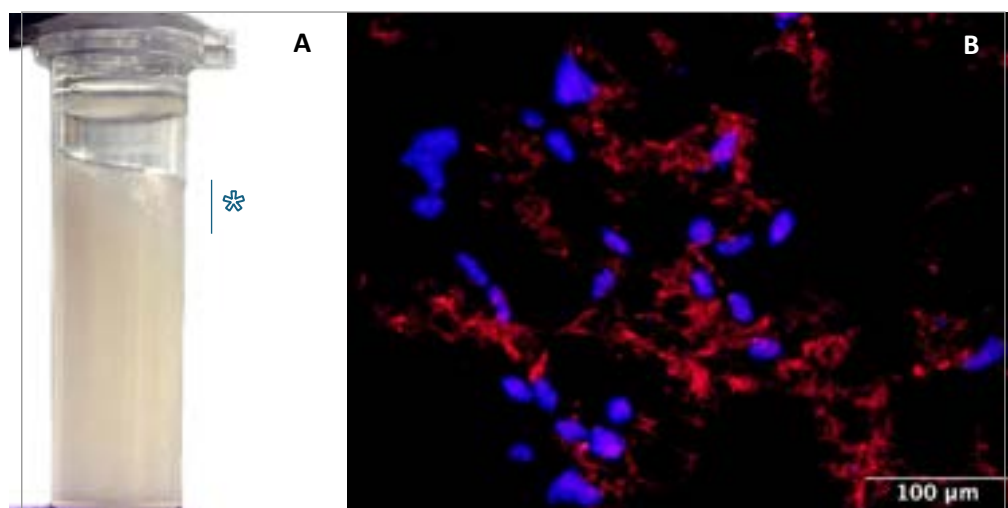
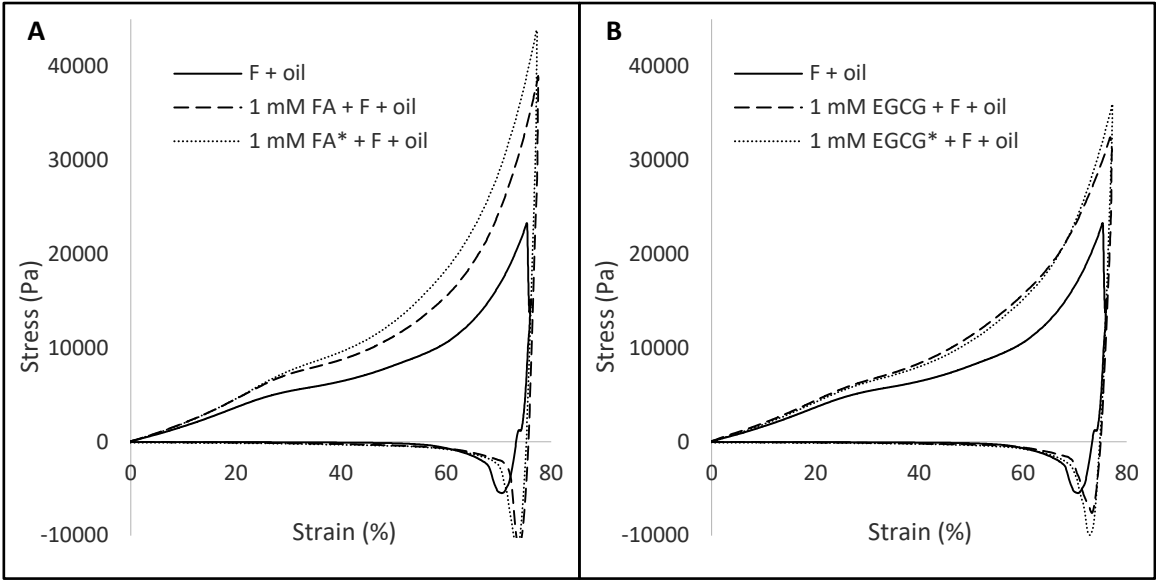


Figure 8

5 min					45 min					90 min				
A	1	2	3	4	1	2	3	4	1	2	3	4		
														
B	1	2	3	4	1	2	3	4	1	2	3	4		
														



Declaration of interests

☒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.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: