



Properties of Laccase mediated sugar beet pectin and Fractionated sugar beet pectin

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ABSTRACT

SBP (Sugar beet pectin) is well known as good emulsifier due to presence of protein and ferulic acids. Ferulic acid in SBP is potential substrates for laccase, an oxidative enzyme, and laccase catalyze formation of SBP homo-conjugates. Using isopropanol fractionation, Fraction 1, which precipitated at the lowest isopropanol concentration, had greater protein and ferulic acid, and Fraction 4, which precipitated at about 40% isopropanaol, showed the best emulsion stability; Fractions 1 and 4 were selected to characterize in this study. Conjugation of SBP and characteristics of protein and ferulic acid rich SBP fractionation were identified by multi angle laser light scattering, refractive index, and UV detection. Laccase treated SBP had larger molecular weight, and more compact, branched structure. During 30 days storage, emulsions prepared with homo-conjugates of SBP stabilized emulsions retained smaller (*d*₄₃) and more uniform particle size (light microscopy) than non-conjugated SBP. Fraction 4 showed the highest UV peak at 280 nm and 325 nm, followed for protein and ferulic acid contents, respectively. Fractionated SBP showed more accumulated fractions at smaller Mw and less accumulated fraction at smaller RMS.

INTRODUCTION

SBP (Sugar beet pectin) is different from non-beet pectin in that it has higher amounts of hydrophobic acetyl groups, ferulic acid and protein linked to the hairy regions.¹ SBP plays a key role as an emulsifying agent since protein and ferulic acids give hydrophobic properties adsorbing on to the oil droplets and carbohydrate impart as stabilizer increasing the viscosity in the aqueous phase.² Oxidative cross-linking between ferulic acid in SBP by laccase, oxidative enzyme, improves gelling ability.^{3,4} It has been known that covalently cross-linked polymers showed improved functionality⁵⁻⁷ and they are more stable over a wide range of pH, ionic strength, heat, proteolysis and other process conditions^{5, 8, 11-12}. We hypothesized that the emulsification of SBP could be improved by enhancing the hydrophobic moiety, attributed to protein and ferulic acids, by isopropanol fractionation. However, while Fraction 4 showed better emulsion stability, Fraction 1 showed the most protein and ferulic acids contents. Therefore, we characterized selected fractionats of SBP (Fraction 1 & Fraction 4) by multi angle light scattering in this study. Additionally, laccase mediated SBP conjugation was characterized and emulsion stability was studied.

MATERIALS AND METHOD

Laccase mediated SBP and emulsion preparation

SBP dispersions (Herbstreith& Fox KG, Elmsford, NY) were prepared by dispersing 3 % (w/v) dry power in 100 mM sodium phosphate buffer (pH 6.5) and centrifuged for 30 min at 5,000 g. Centrifuged SBP were equilibrated with and without laccase (*Rhusvernificera*, EC 1. 10. 3. 2, Sigma – Aldrich), 30 °C for 20 hr. Tetradecane, 10 % (Fisher Scientific, NJ) was premixed with 1 % wt laccase treated or control SBP using a PRO300A homogenizer (Pro Scientific Inc., Oxford, Ct) for 1 min, and immediately homogenized on ice by 3 passes at 40 MP in a high pressure homogenizer (Emulsi-Flex C5, Avestin Inc., Ottawa, ON, CA).

Isopropanol fractionated SBP preparation

An aliquot of 100 ml isopropanol (10 %) was added to 900 ml of 2 % (w/v) SBP dispersion (GENU® pectin type BETA, CPKelco, Denmark) and centrifuged at 4°C and 8,000 g for 20 minutes. The supernatant was separated from the pellet by filtration using Miracloth™ (Calbiochem, La Jolla, CA). The pellet was dried under a fume hood to evaporate residual isopropanol. Additional aliquots of 100 mL isopropanol were sequentially added to the supernatant, centrifuged and dried. This process was repeated until no further pellet was obtained. The 6 dried pellets were collected and was ground using a Satake AC 100 grinder (Stafford, TX). Fraction 1 and 4 were selected to do assay in this study due to varying amounts of protein and ferulic acid contents.

Characterization.

Laccase mediated SBP conjugation was assessed using size exclusion chromatography with a multi-angle light scattering detector (DAWN DSP-F), a UV detector and a refractive index concentration (OptiLab) detector. Emulsions were stored 30 days at 30 °C and particle size (Model MSS, Malvern Instruments Limited, Worcestershire, U.K.) and light microscopy (Leica DM LB, Leica Microsystems Inc., Bannockburn, USA). SBP and Fractions 1 and 4 were characterized using Heleos II, Optilab TrEX, and UV detectors at 280 nm (protein) and 325 nm (ferulic acid) to quantify protein and ferulic acid, respectively. . Elution data was assessed by Astra 6 software.

RESULTS & DISCUSSION

Laccase mediated homo-conjugation of SBP

Table 1. Mw, RMS and recovery % of SBP and L-SBP. ¹

	Peak	MW (kDa)	RMS (nm)	Recovery %
SBP	1 st	372.7±14.1 ^a	28.4 ± 1.6 ^a	43.4 ±7.0 ^a
	2 nd	65.7±13.2 ^a	36.7 ± 2.3 ^a	9.7 ± 1.1 ^a
L-SBP	1 st	403.2±23.7 ^b	23.3 ± 1.7 ^b	32.2 ± 4.5 ^b
	2 nd	83.2±24.3 ^b	28.3 ± 2.2 ^b	6.8 ± 1.5 ^a

¹ Means with different superscripts for 1st or 2nd peak are significantly different (p<0.05) .

SBP eluted as two incompletely resolved peaks detected by HPSEC-MALLS. L-SBP shows a smaller RMS radius, larger Mw than SBP in both the first and second peak (p<0.05) indicating that conjugated L-SBP is more compact and has a more branched structure than control SBP.

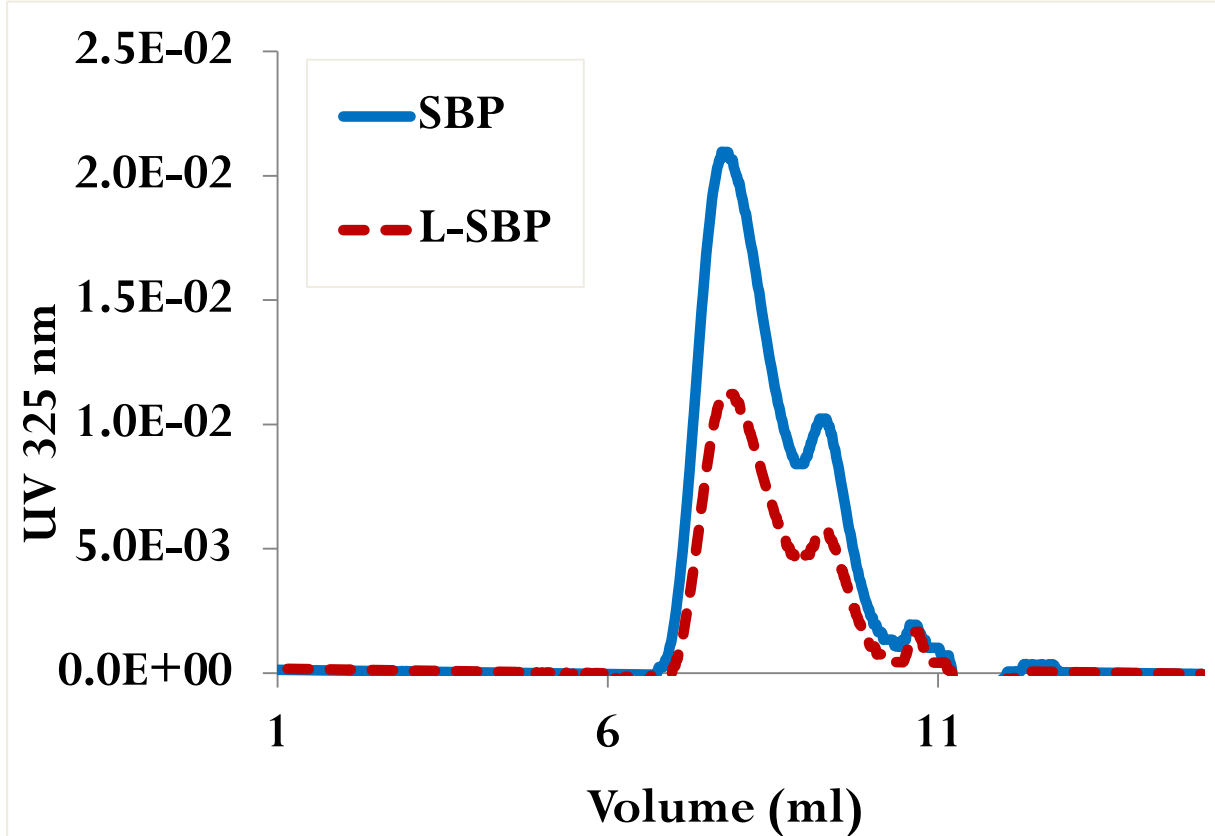


Fig.1. UV₃₂₅ elution of SBP and L-SBP

Laccase catalyzed cross-linking of SBP is confirmed by decrease in ferulic acid absorbance at UV₃₂₅ .

Emulsification of SBP and laccase treated SBP

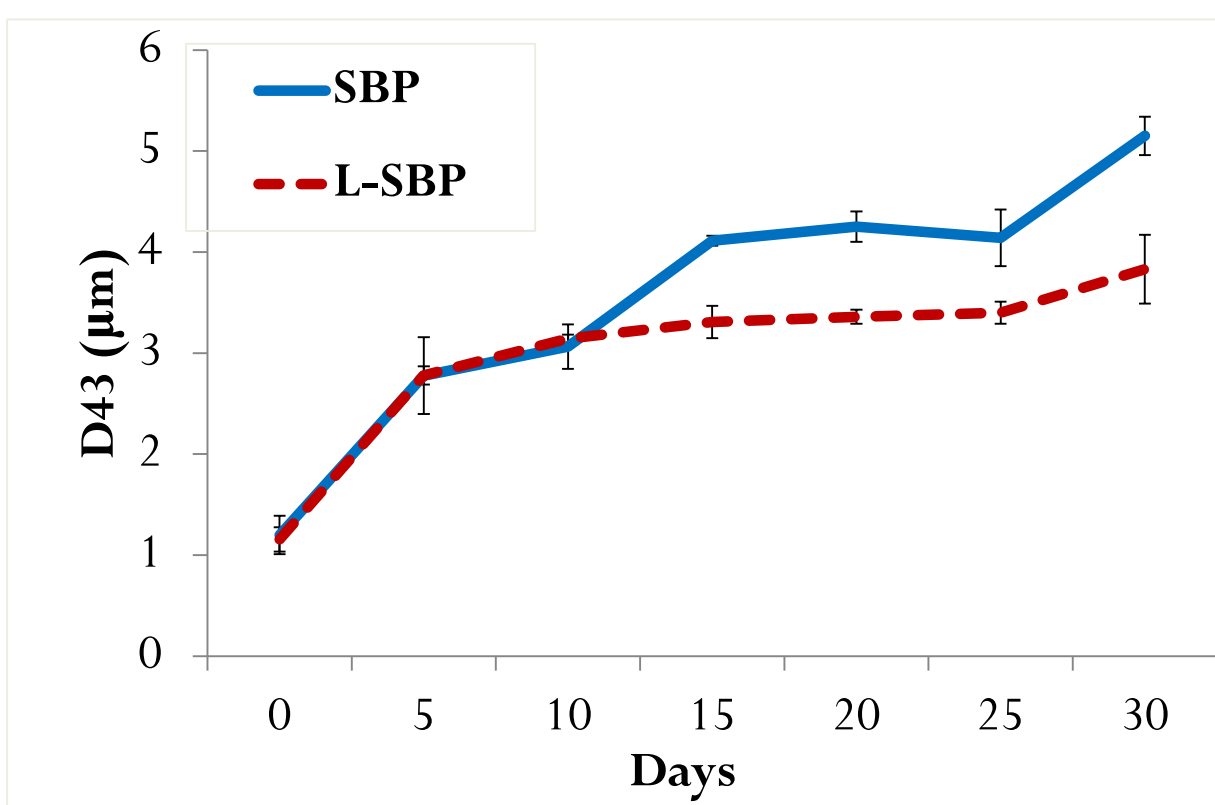


Fig. 2. *d*₄₃ diameter of SBP and L-SBP emulsions for 30 incubation days.

Emulsion droplets of SBP and L-SBP emulsions had almost the same size at 0 day. However, noticeably large droplets and some flocculation were observed at 30 days in SBP emulsion whereas L-SBP emulsion droplets were still small and well dispersed.

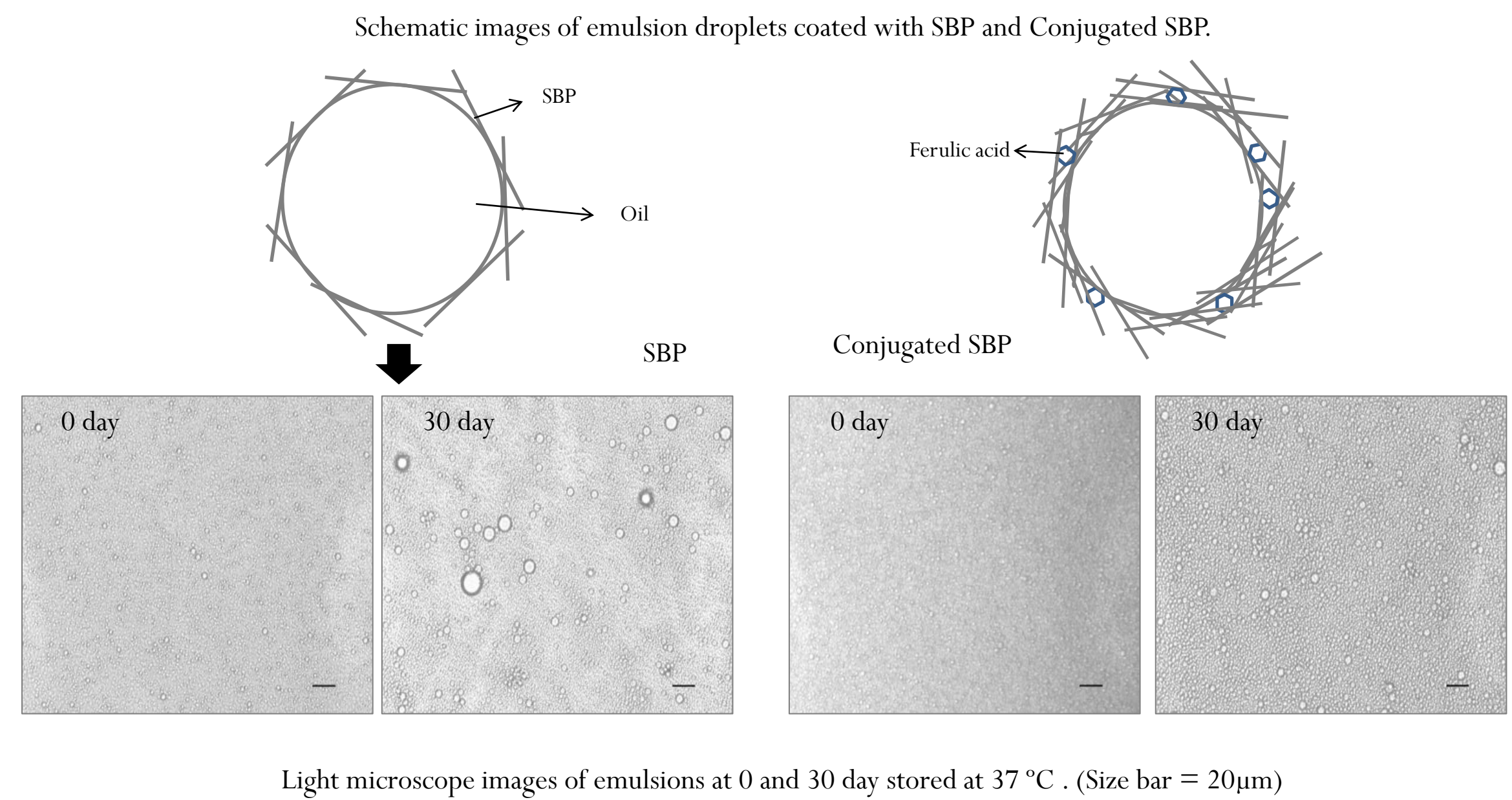


Fig 3. Light microscopy images of SBP,L-SBP emulsions at 0 and 30 day. Size bar = 20µm.

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Emulsion droplets of SBP emulsions have noticeably large droplets and some flocculation at 30 days whereas L-SBP emulsion droplets were still small and well dispersed.

Properties of fractionated SBP

Table 2. Mw, RMS and recovery % of SBP and fractionated SBP

	Slope	Mw (kDa)	Rn (nm)	Rw (nm)	Rz (nm)	Recovery (%)
SBP-con	0.21±0.01	429.58±20.2	50.75±14.2	50.43±19.0	51.48±19.0	67.68±4.1
Frac1	0.14±0.02	380.95±49.8	64.90±15.4	62.95±8.28	62.22±8.3	31.58±4.8
Frac4	0.18±0.01	527.68±22.4	46.78±3.3	45.54±3.0	48.60±2.8	78.70±1.5

Fractionated SBP show smaller conformational plot which means more branched. Fraction 1 has the smallest MW and bigger RMS. On the contrary, Fraction 4 has the biggest MW and the smallest RMS.

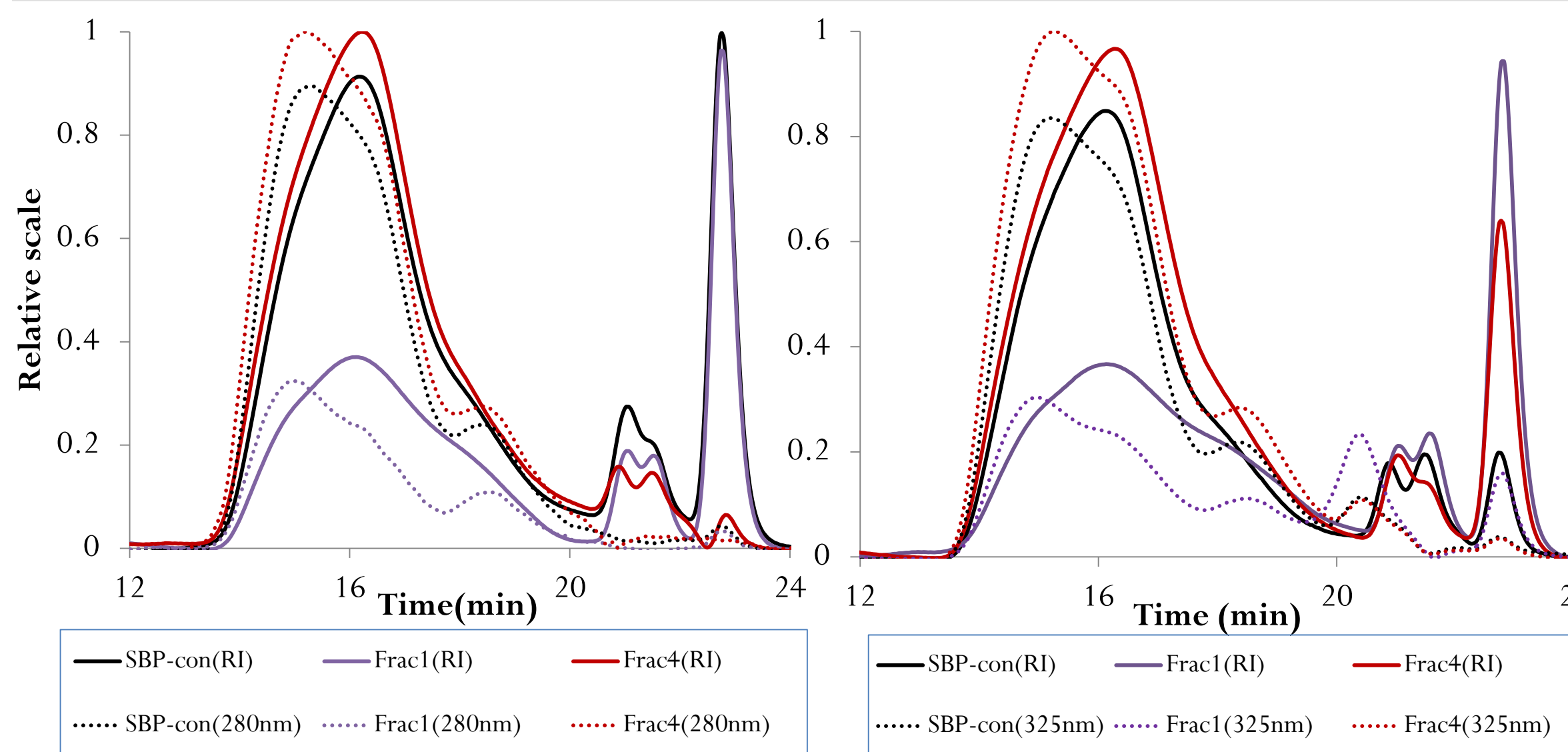


Fig. 4. UV elution profile of SBP and Fractionated SBP at 280 nm(a) and 325 nm(b).

SBP and SBP fractions eluted as a polydisperse polymer in up to 4 peaks. UV absorbing material (protein) was associated with higher MW peaks; small weight material was observed in SBP, Fraction 1 and Fraction 4. Fraction 1 shows the lowest UV peak at earlier elution times, most likely associated with low recovery .

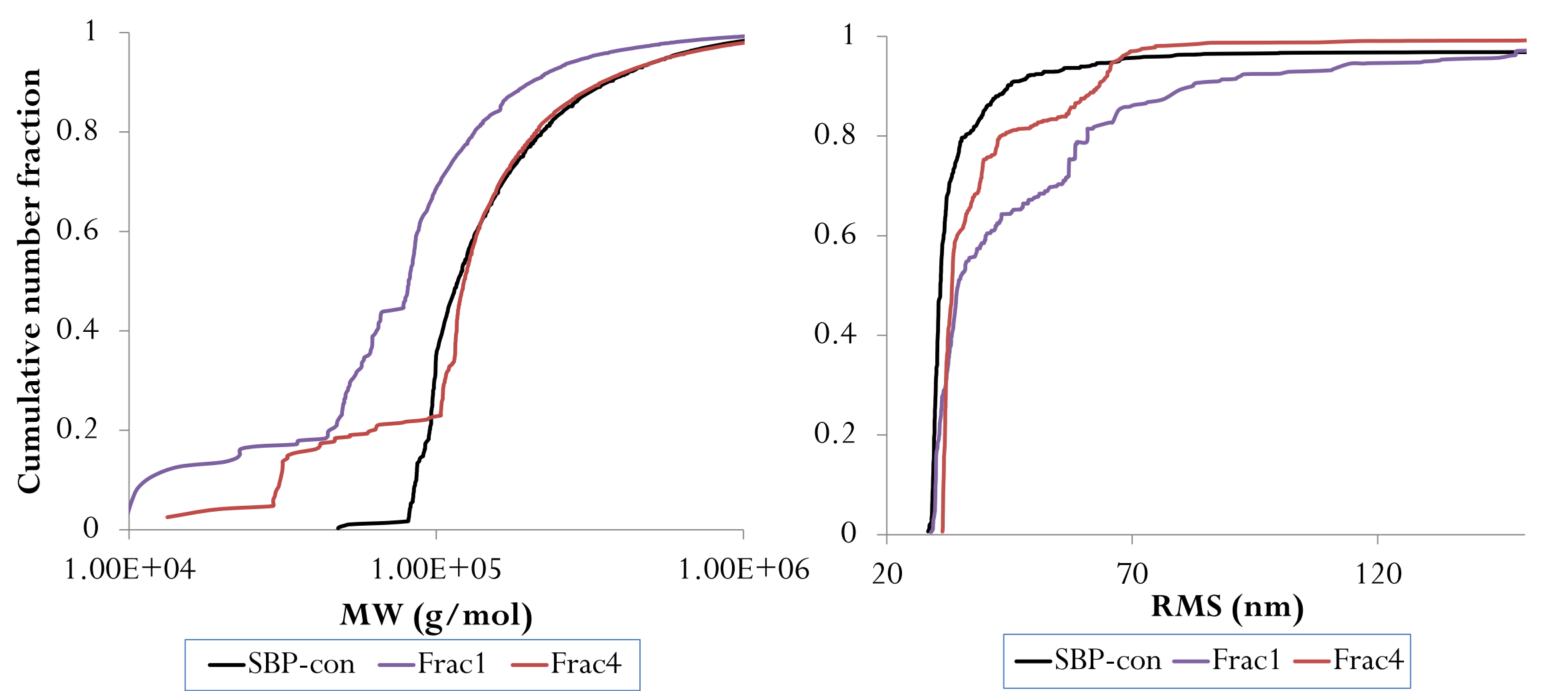


Fig.5. Cumulative number fraction against MW (a) and RMS (b) of SBP and Fractionated SBP.

Fractions 1 and 4 were higher in lower MW pectin than control SBP. On the other hand, the RMS was higher in Fractions 1 and 4 than control SBP. Results suggest that lower MW SBP fractions are less linear, more branched. Fractionated SBP showed more fractions at lower molecular weight and less fraction at smaller RMS.

CONCLUSIONS

Laccase mediated SBP conjugates had a different shape and enhanced the emulsion stability by formation of a more compact and thicker layer. SBP control and isopropanol fractionated SBP Fraction 1 and 4 differed by MW, RMS, primarily in low MW pectins. There are protein and ferulic acids in SBP that do not contribute to emulsion activity or stability.

Reference available on request..