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A STUDY ON THE EFFECT OF CINNAMON AND CINNAMALDEHYDE ON BOVINE SERUM ALBUMIN (BSA) PROTEIN BY USING FT-RAMAN SPECTROSCOPY

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ABSTRACT

FT-Raman spectroscopy revealed comparative intensity changes of many bands and wave number shifts, when comparing bovine serum albumin (BSA) separately, BSA mixed with 500 ppm cinnamaldehyde and BSA mixed with 500 ppm cinnamon. It is clear that there are small shifts in wave numbers. The intensity of tryptophan band at 760 ± 2 cm^{-1} was 0.42 ± 0.02; there was no significant difference between the value for BSA alone and BSA + cinnamon sample (0.433 ± 0.081). In contrast, in sample BSA + cinnamaldehyde the tryptophan band intensity (0.296 ± 0.066) significantly decreased (p<0.05). The decrease was ascribed to Trp vibrational modes and C-H bending due to the enhancement of hydrophobic residues open to the outer surface of proteins. This result can be used to monitor denaturation in protein aliphatic and aromatic side chains. The decrease in the tyrosine ratio 855/830 cm^{-1} of the BSA mixture with cinnamon and cinnamaldehyde separately, compared to BSA alone, was attributed to strong hydrogen bonding interactions in the mixture involving tyrosine residues.

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1 Introduction

Proteins are significant functional polymers that are widely used in biochemical, pharmaceutical and food applications (Damodaran, 1996). The alteration of proteins via lipid peroxidation products, together with free radicals and aldehydes, has been thoroughly studied (Uchida, 2003; Ozeki et al., 2005). The product that results from aldehyde lipid oxidation causes a strange flavour, a rancid odour and the discoloration of meat (Fogetty et al., 1989). Lipid peroxidation releases high amounts of various by-products (Paliyath et al., 2011), such as malondialdehyde, and bioactive α,β-unsaturated aldehydes, including 4-hydroxy-2-trans-nonenal and hydroxynonenal, are regularly considered to be indicators of in vivo oxidative stress (Refsgaard et al., 2000). However, Ulberth & Roubicek (1995) and Gary & Monahan (1992) reported that hexanal is a final lipid peroxidation product that is usually recognized as a marker of food quality.

The interaction mechanism between proteins and polyphenols seems to have a non-covalent nature whenever protein molecules are attached together via polyphenolic compounds that act as bridges (Siebert et al., 1996). Further, Ozdal et al. (2013) have reported the consequences of protein-phenolic interactions on the structure and functionality of protein and phenolic compounds under a range of conditions. They reported that the main factors such as temperature, protein type, concentration, pH and the structure and nature of the phenolic compounds affect interactions of proteins with polyphenols. Damodaran (1996) reported that the extremely complex polymers formed by proteins and polyphenols are fabricated using twenty amino acids. Amino acids are comprised of an amino group, a carboxyl group a hydrogen atom, and side-chain R groups that are attached covalently to the α-carbon atom (Figure 1).
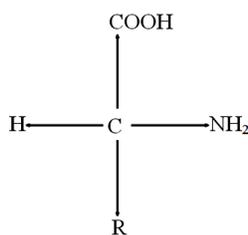

Figure 1 The General Structure of an amino acid

2 Materials and methods

2.1 Materials

Deuterium oxide (D_2O), bovine serum albumin (BSA) lyophilized powder, crystallized ≥98.0% (GE) and cinnamaldehyde liquid (Kosher natural 95%) were procured from Sigma Aldrich, Poole, UK. Powdered cinnamon was purchased from a local market (Guildford).

2.2 Methods

2.2.1 Sample preparation

One and a half mg of cinnamon or cinnamaldehyde (500 ppm) was added to 3g BSA that was dissolved in 20 ml of D_2O (15% BSA).

2.2.2 FT-Raman spectroscopy

Samples were placed in 7mL glass containers (FBG-Anchor, Cricklewood, London) in a Perkin- Elmer System 2000 FT-Raman spectrophotometer with excitation from a Nd: YAG laser at 1064 nm. The instrument was calibrated for frequency using the sulphur line at 217cm^{-1}. The samples were analysed in triplicate using laser power of 1785 mW. The spectra were an average of 64 scans; the baseline was corrected, scans were smoothed and normalised to the intensity of the phenylalanine band at 1004 cm^{-1} (Howell et al., 1999; Li-Chan et al., 1994). The spectra were analysed using Grams 32 (Galactic Industries Corp., Salem, NH). Using literature values, assignments of the bands in the spectra to protein vibrational modes were made (Howell et al., 1999; Careche et al., 1999; Li-Chan et al., 1994). Since the intensity of phenylalanine shows a strong band at 1004 cm^{-1} and it is known to be unaffected by changes in the microenvironment, this band was used as an internal standard for normalisation (Tu, 1986). The results are presented as mean ± standard deviation for relative peak intensity of the spectra bands.

2.3 Statistical Analysis

The mean value and standard deviation were calculated for all experiments. A one-way ANOVA analysis with a Bonferroni test and a Graphpad Prism 6 computer program were used to calculate the differences between the samples in terms of FT-Raman and rheology.

3 Results and Discussion

Novel Raman structural markers were anticipated for the tryptophan and histidine side chains by examining the spectra-structure interactions of the model compounds. Raman structure is used to provide information about conformation, hydrogen bonding, hydrophobic interaction, and the ionization of the indole ring of Trp. Figure 2 illustrates the Raman spectrum of BSA alone (black line) in D_2O, BSA mixed with 500 ppm cinnamaldehyde (blue line) in D_2O and BSA mixed with 500 ppm cinnamon (red line) in D_2O. It is clear that there are relative intensity changes in many bands and also small shifts in wave numbers. Trp residue is found at the 760 cm^{-1} band and has been widely investigated (Taukeuich, 2003).
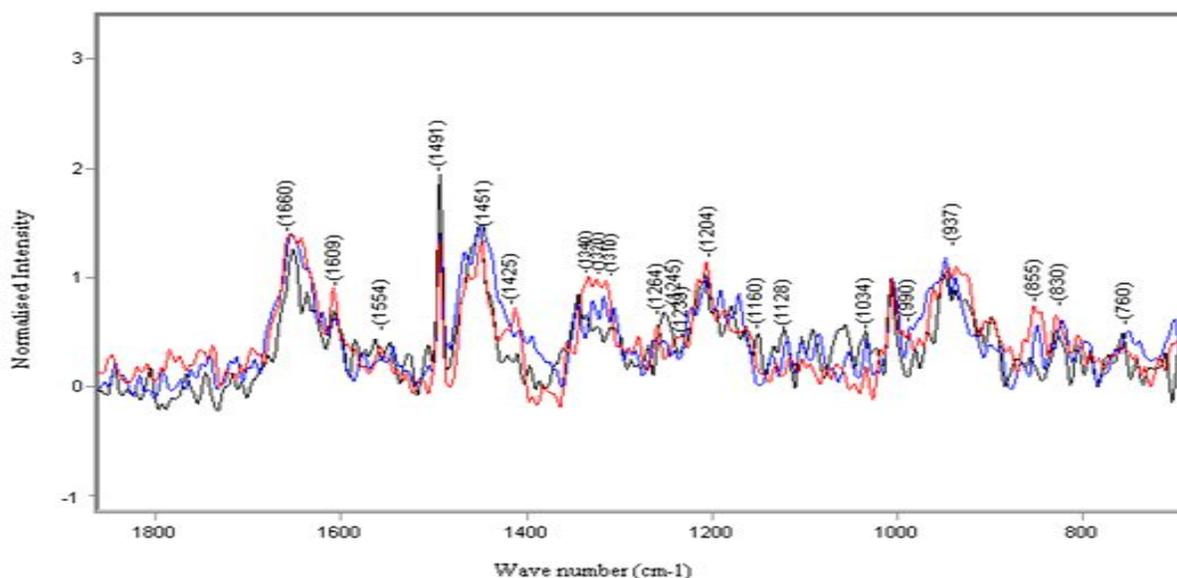
Figure 2 FT-Raman spectra (700-1800 cm^{-1}) of 15% BSA in D_2O (black line), 15%BSA + 500 ppm cinnamaldehyde in D_2O (blue line) and 15%BSA + 500 ppm cinnamon in D_2O (red line). Data represent the mean ± SD.

Trp contains an N atom, which plays a significant role in hydrogen bonding. The hydrophobic groups and the proton donor site of the indole ring are essential for the interaction with other amino acids. Consequently, any change in amino acids, for instance, lys (lysine), leads to a change in the intensity of the tryptophan band. The intensity of the tryptophan band near 760 ± 2 cm^{-1} for BSA was found to be 0.42 ± 0.02; there was no significant difference between the intensity band of the BSA + cinnamon sample (0.433 ± 0.081) and BSA. However, for the BSA + cinnamaldehyde sample, the tryptophan intensity band was significantly ($p<0.05$) diminished (0.296 ± 0.066) (Table 1; Figure 2). It is quite clear that the cinnamaldehyde treated sample demonstrated a considerable effect (Figure 2). However, the diminishing of the tryptophan band in the cinnamaldehyde sample could be associated with the effect on hydrophobic residues during the interaction.
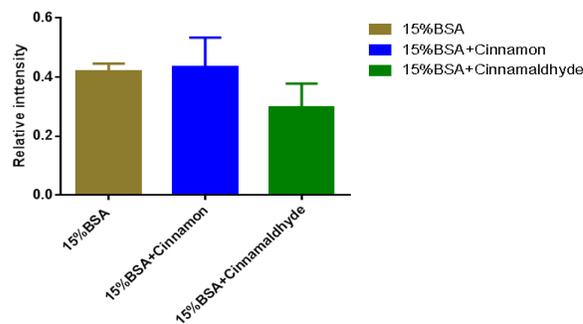
Figure 3 illustrates the tryptophan band intensities at 760 cm^{-1} for 15% BSA, 15% BSA + 500 ppm cinnamon and 15%BSA + 500 ppm cinnamaldehyde in D_2O. Although the results reveal insignificant band intensity differences between BSA, BSA plus cinnamon and BSA plus cinnamaldehyde, an obvious reduction was noticed in the band for the BSA + 500 ppm cinnamldehyde mixture, compared to the two other samples (Figure 3).
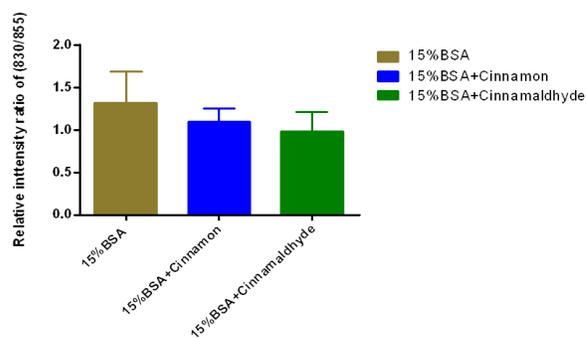
Two strong peaks appeared at 855 and 830 cm^{-1}, revealing the presence of tyrosine. The tyrosine doublet ratios for 15% BSA, 15% BSA + 500 ppm cinnamon and 15% BSA + 500 ppm cinnamaldehyde in D_2O are represented in Figure 4. There were slight differences in the tyrosine doublet ratio: 855/830

Figure 3 Tryptophan intensity band at 760 cm^{-1}
(Data are expressed as the mean ± SD, n=3)

Figure 4 Tyrosine doublet ratio 855/830 cm^{-1}
(Data are expressed as the mean ± SD, n=3)

Table 1 Relative intensity values (mean ± SD) in selected regions of the FT-Raman spectra (760-2976 cm-1) of 15% BSA,
15% BSA + cinnamon and 15%BSA + 500 ppm cinnamaldehyde.

Assignments and wave Number (cm⁻¹ ±4)	15% BSA	15% BSA+ cinnamon	15% BSA + cinnamaldehyde
Trp (760)	0.42±0.02	0.433±0.081	0.296±0.066
Tyr (830, 855) 855/830	0.809±0.22	1.05±0.18	0.921±0.12
Helix C-C stretch, CH₃ symmetric stretch (937)	0.937±0.051	1.09±0.024	1.06±0.075
β-sheet type structure (990)	0.295±0.013	0.423±0.064	0.328±0.053
Phe, ring band (1034)	0.522±0.107	0.197±0.026	0.318±0.092
Isopropyl ant symmetric stretch CH stretch back bone (1128)	0.396±0.011	0.233±0.02	0.407±0.087
CH₃ anti symmetric (aliphatic) CH₃ rock (aromatic) (1160)	0.521±0.062	0.555±0.024	0.463±0.039
β-sheet type (1239)	0.349±0.095	0.487±0.074	0.388±0.073
Amide III (random coil) (1245)	0.493±0.091	0.37±0.034	0.357±0.063
Amide III (1264)	0.343±0.021	0.608±0.045	0.474±0.078
Amide II (1320)	0.53±0.042	1.09±0.111	0.737±0.063
C-H bending doublet from trp (1340)	0.806±0.028	1.084±0.192	0.677±0.096
(sh*, residue vibration) asp, glu, lys (1425)	0.293±0.026	0.642±0.081	0.611±0.09
Aliphatic groups, CHbend (1451)	1.421±0.068	1.574±0.222	1.261±0.156
Trp (1554)	0.22±0.057	0.391±0.018	0.331±0.034
Amide I (1660)	1.259±0.063	1.386±0.117	1.033±0.22
CH stretch, aliphatic (2940)	3.647±0.144	4.155±0.256	3.45±0.326
Shoulder (2888)	1.679±0.155	2.035±0.035	1.669±0.141
Shoulder (2976) (2969)	1.51±0.093 1.833±0.19	2.113±0.017 2.426±0.039	1.498±0.177 1.727±0.067

Tyr (tyrosine), Trp (tryptophan), Phe (phenylalanine); The spectra were an average of 64 scans baseline corrected, smoothed and normalized to the intensity of Phe band at 1004 cm⁻¹

cm⁻¹ for the BSA solution (1.321 ± 0.31) as compared with the BSA mixed with cinnamaldehyde and cinnamon (0.987±0.19 and 1.103 ± 0.13, respectively (Table 1 and Figure 4). The results show the increased buriedness or linking of tyrosine residues, which are strong hydrogen bond donors in the mixture (Nakai et al., 1994).

In Figure 5 a strong peak at 937 cm⁻¹ (α-helix region) and high β-sheet content occur in the untreated BSA sample. There was no significant difference (P > 0.05) in peak intensity in the cinnamon- and cinnamaldehyde-treated samples (1.09 ± 0.024 and 1.06 ± 0.075, respectively) as compared to 0.937 ± 0.051 for BSA only (Figure 5). Similar results were reported by Ngarize et al. (2004) for heated BSA. There was a weak intensity peak at 990 cm⁻¹, representing the beta sheet structure (Figure 2). Table 1 also shows that the non-treated BSA sample's peak intensity at 990 cm⁻¹ was 0.295 ± 0.013, which is significantly lower than those of
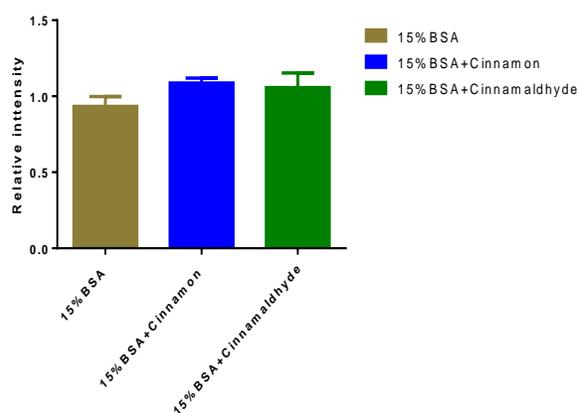

Figure 5 α-helix regions at 937cm⁻¹
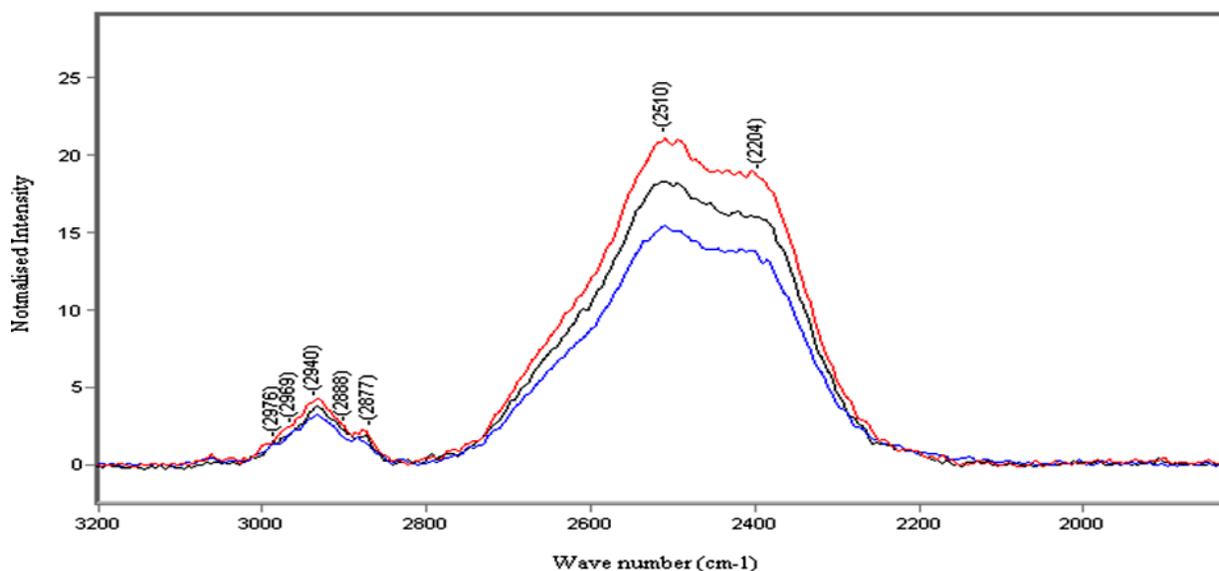(Data are expressed as the mean ± SD, n=3)

Figure 6 FT-Raman spectra (1800-3200 cm^{-1}) of 15% BSA (black line), 15%BSA + 500 ppm cinnamaldehyde (blue line) and 15%BSA + 500 ppm cinnamon (red line) in D$_2$O (Data represent the mean ± SD)

the cinnamon and cinnamaldehyde treated BSA samples (0.423 ± 0.064 and 0.328 ± 0.053, respectively) (P < 0.05). This increase suggests the existence of anti-parallel β-pleated sheets (Ngarize et al., 2004). The absence of a peak at 1239 cm^{-1} was noticed (Figure 2), together with the presence of a peak at 1320 cm^{-1}, indicating greater helical structure content in egg albumen, in agreement with Mine et al., (1990). The band at 1340 cm^{-1} is due to C-H bending from tryptophan. A significant (p<0.05) decrease in band intensity was noticed in the cinnamaldehyde treated BSA sample (0.677 ± 0.096) as compared to the untreated BSA sample (0.806 ± 0.028) (Table 1). This decrease was attributed to the exposure of hydrophobic residues to cinnamaldehyde. A similar result was reported by Ngarize et al. (2004) during the heating of BSA. Also, Badii & Howell (2002) have reported this drop in peak intensity associated with the aggregate formation of actomyosin in frozen cod and haddock. This finding implies that changes in the hydrophobic environment around the aliphatic and aromatic side chains can be used to screen for denaturation and conformational changes in proteins (Ngarize et al., 2004).

Figure 6 reveals the FT-Raman spectra in the 1800-3200 cm^{-1} region for 15% BSA, 15% BSA + 500 ppm cinnamaldehyde and 15% BSA + 500 ppm cinnamon in D$_2$O. The broad peak between 2000 and 2600 cm^{-1} is attributed to D$_2$O. The CH stretching band at 2940 cm^{-1} was assigned to aliphatic amino acids, mainly leucine residues (Howell et al., 1999). The vibrations at (2988 sh, 2976 sh and 2969 sh, where sh denotes shoulder) are attributed to the asymmetrical and symmetrical stretching of the CH$_3$ and CH$_2$

groups of the aliphatic chains. The intensities of these bands show a slight increase in the BSA mixture with cinnamon.

Figure 7 shows the intensities of the CH bending vibrations for 15% BSA, 15% BSA + 500ppm cinnamon and 15% BSA + 500ppm cinnamaldehyde in D$_2$O. No significant changes in CH bending were noticed after mixing BSA with cinnamon or cinnamaldehyde antioxidants. However, a slight increase in the CH bending intensity was noticed in the BSA mixture with cinnamon, while a decrease in the intensity was noticed in the case of the BSA mixture with cinnamaldehyde.
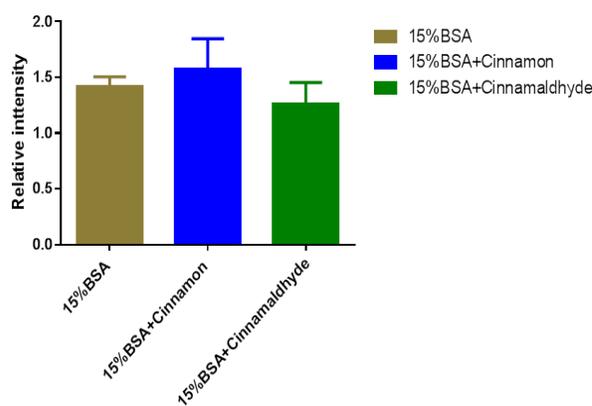

Figure 7 Aliphatic CH bending vibration at 1454 cm^{-1})
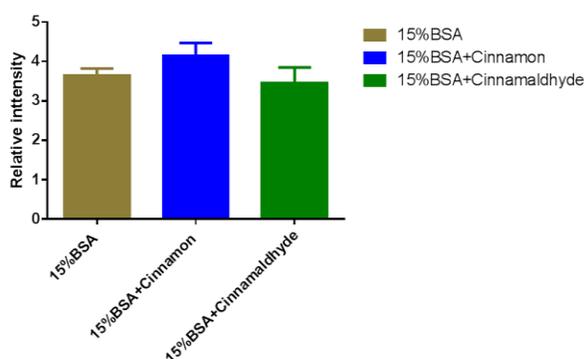(Data are expressed as the mean ± SD, n=3)

Figure 8 CH stretching vibration at 2940 cm^{-1}
(Data are expressed as the mean $\pm$ SD, n=3)

Changes in the CH stretching band at 2940 cm^{-1} are represented in Figure 8. No significant changes in the CH stretching intensity were noticed when comparing BSA and the BSA mixture with either cinnamon or cinnamaldehyde. A slight increase in intensity in the sample treated with cinnamon was noticed, and a drop in the intensity was recorded for the BSA mixture with cinnamaldehyde. Changes in the CH band stretching follow the same intensity trend change as CH bending, confirming the active role of cinnamon and cinnamaldehyde in the conformational rearrangements of proteins.

Changes in CH bending (1450 cm^{-1}) and stretching (2940 cm^{-1}) suggest changes in the aliphatic side chains of BSA proteins. The increase for the cinnamon BSA mixture suggests the participation of aliphatic amino acid residues in hydrophobic interactions (Howell & Li-Chan, 1996). Similar results have been reported by Howell et al. (2001) when they studied lysozyme-corn oil protein-lipid interactions.

Conclusion

The study shows that protein-polyphenol (500 ppm cinnamon and cinnamaldehyde) interactions occurred mainly via aliphatic, aromatic and tyrosine amino acids.

Acknowledgements

Conflict of Interest

Authors would hereby like to declare that there is no conflict of interests that could possibly arise.

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