

The interaction of milk protein and black tea aroma compounds: Selectivity, binding mechanism, and theaflavins' effect

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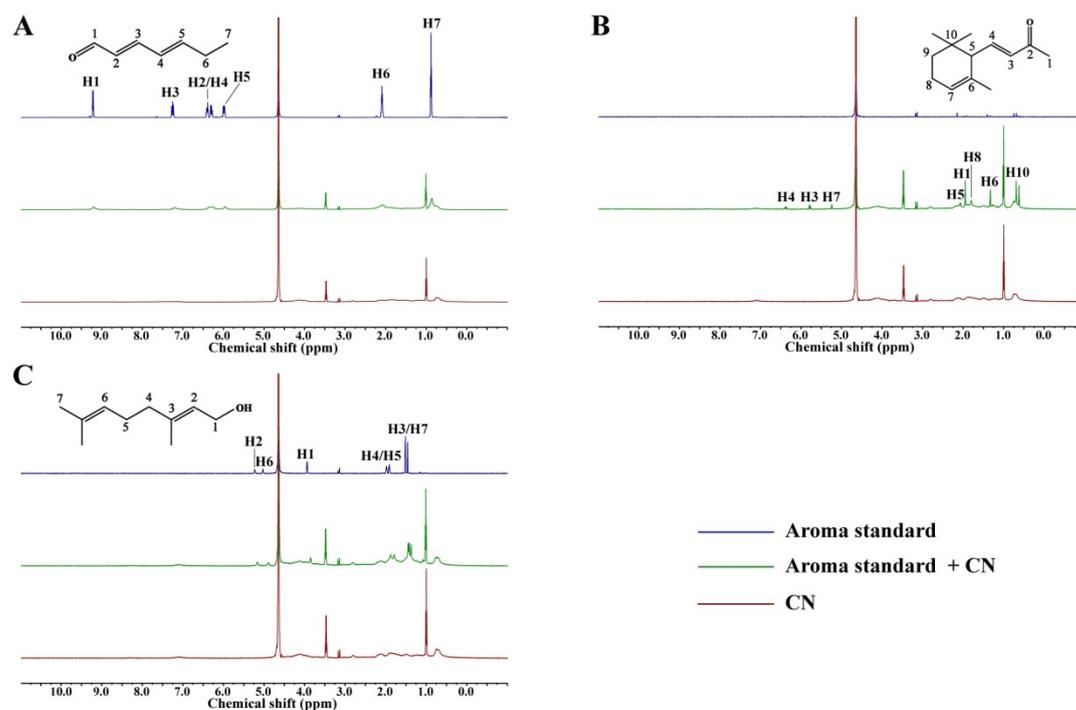


Fig. S1 ¹H-NMR spectra of aroma compounds mixed with CN ((A) (*E,E*)-2,4-heptadienal; (B) α -ionone; (C) geraniol).

Table S1 Samples information for various black teas and milk.

| Material | Abbreviation | Place of production | Date of manufacture |
|----------------------------------|--------------|---------------------|---------------------|
| Pure milk from Yili | / | China | 2024.05 |
| Indian black tea <i>No.1</i> | I-1 | India | 2022.04 |
| Indian black tea <i>No.2</i> | I-2 | India | 2021.04 |
| Indian black tea <i>No.3</i> | I-3 | India | 2023.07 |
| Chinese black tea <i>No.1</i> | C-1 | China | 2023.11 |
| Chinese black tea <i>No.2</i> | C-2 | China | 2022.08 |
| Chinese black tea <i>No.3</i> | C-3 | China | 2023.01 |
| Chinese black tea <i>No.4</i> | C-4 | China | 2023.07 |
| Turkish black tea <i>No.1</i> | T-1 | Turkey | 2022.06 |
| Turkish black tea <i>No.2</i> | T-2 | Turkey | 2021.11 |
| Turkish black tea <i>No.3</i> | T-3 | Turkey | 2022.10 |
| Kenyan black Tea <i>No.1</i> | K-1 | Kenya | 2023.02 |
| Kenyan black Tea <i>No.2</i> | K-2 | Kenya | 2023.01 |
| Kenyan black Tea <i>No.3</i> | K-3 | Kenya | 2023.05 |
| Sri Lankan black Tea <i>No.1</i> | S-1 | Sri Lanka | 2022.07 |
| Sri Lankan black Tea <i>No.2</i> | S-2 | Sri Lanka | 2023.01 |
| Sri Lankan black Tea <i>No.3</i> | S-3 | Sri Lanka | 2022.08 |

Table S2 ¹H NMR spectral information of 7 aroma compounds and CN.

| Sites | Chemical shift (ppm) | | Peak pattern | |
|--------------------------------|----------------------|-------------------|----------------|-------------------|
| | Aroma standard | CN+aroma standard | Aroma standard | CN+aroma standard |
| Benzaldehyde | | | | |
| H1 | 9.76 | 9.72 | s | s |
| H2/H6 | 7.78 | 7.73 | d | s |
| H3/H5 | 7.59 | 7.53 | t | s |
| H4 | 7.45 | 7.40 | t | s |
| H4 | / | 6.99 | / | d |
| β -Ionone | | | | |
| H3 | / | 5.81 | / | d |
| H1/H7 | 2.20; 1.96 | 1.98; 1.80 | s;s | s; s |
| H6/H8 | 1.46; 1.33 | 1.36; 1.49 | s;s | s |
| H9 | / | 1.22 | / | s |
| H10 | 0.92 | 0.81 | s | s |
| Methyl salicylate | | | | |
| H6 | 7.76 | 7.12 | d | d |
| H4 | 7.40 | 6.79 | t | t |
| H5 | 6.85 | 6.40 | m | d |
| H3 | / | 6.21 | / | t |
| H1 | 3.79 | 3.24 | s | s |
| Linalool | | | | |
| H2 | 5.80 | 5.70 | dd | d |
| H1/H7 | 5.01 | 4.55 | m | m |
| H5 | 1.84 | 1.79 | t | s |
| H8 | 1.50 | 1.44 | s | s |
| H4 | 1.40 | 1.37 | dd | s |
| H3 | 1.12 | 1.04 | s | s |
| (<i>E,E</i>)-2,4-heptadienal | | | | |
| H1 | 9.22 | 9.21 | dd | s |
| H3 | 7.25 | 7.21 | dd | s |
| H2/H4 | 6.40 | 6.31 | m | d |
| H5 | 5.98 | 5.96 | m | s |
| H6 | 2.09 | 2.07 | m | s |
| H7 | 0.88 | 0.86 | t | s |
| α -Ionone | | | | |
| H4 | 6.71 | 6.37 | m | dd |
| H3 | 5.99 | 5.78 | d | d |
| H7 | 5.44 | 5.24 | s | s |
| H5 | 2.15 | 2.07 | s | d |
| H1 | 1.95 | 1.95 | s | s |
| H8 | 1.90 | 1.80 | s | s |

| | | | | |
|----------------------------|------|------|-----|---|
| H6 | 1.40 | 1.33 | s | s |
| H10 | 0.69 | 0.66 | d | d |
| ----- Geraniol ----- | | | | |
| H2 | 5.23 | 5.16 | t | s |
| H6 | 5.03 | 4.89 | t | s |
| H1 | 3.94 | 3.85 | d | d |
| H4/H5 | 1.84 | 1.88 | t,t | d |
| H3/H7 | 1.49 | 1.4 | d,s | t |

Note: “s”: singlet; “d”: doublet; “t”: triplet; “t”: multiplet; “dd”: doublet of doublets.

Table S3 Binding parameters and molecular docking scores of four types of casein with different aroma compounds.

| Component | | Binding energy (kcal/mol) | Binding sites |
|----------------|--------------------------------|------------------------------|---------------------------|
| α_1 -CN | Benzaldehyde | -3.24 | Gly 25 |
| | (<i>E,E</i>)-2,4-Heptadienal | -2.82 | Gln 24 |
| | Methyl salicylate | -3.21 | His 23, Gly 25, Leu 26 |
| | β -Ionone | -4.37 | Gln 24 |
| | α -Ionone | -4.34 | Gly 25 |
| | Linalool | -2.98 | Leu 26 |
| | Geraniol | -2.93 | Leu 26 |
| α_2 -CN | Benzaldehyde | -3.30 | Ile 209 |
| | (<i>E,E</i>)-2,4-Heptadienal | -3.22 | Val 58 |
| | Methyl salicylate | -3.74 | Ile 209, Pro 207 |
| | β -Ionone | -4.55 | Asn 122 |
| | α -Ionone | -4.69 | Lys 206 |
| | Linalool | -3.98 | Ile 209, Pro 207 |
| | Geraniol | -3.50 | Try 104 |
| β -CN | Benzaldehyde | -3.26 | Arg 198 |
| | (<i>E,E</i>)-2,4-Heptadienal | -3.84 | Gln 197 |
| | Methyl salicylate | -3.85 | Val 23, Gly 25, Ser 30 |
| | β -Ionone | -4.64 | Lys 44 |
| | α -Ionone | -4.24 | Glu 26 |
| | Linalool | -3.67 | Met 200 |
| | Geraniol | -3.88 | Arg 198 |
| κ -CN | Benzaldehyde | -3.26 | Ile 49 |
| | (<i>E,E</i>)-2,4-Heptadienal | -3.05 | Ile 47 |
| | Methyl salicylate | -3.06 | Leu 71, Asn74 |
| | β -Ionone | -4.34 | Ile 49 |
| | α -Ionone | -4.29 | Asn 74 |
| | Linalool | -3.16 | Leu 71, Asn 74 |
| | Geraniol | -3.08 | Lys 45, Ile47 |

Table S4 The effect of different concentrations of theaflavins on the adsorption aroma

of milk.

| Compounds | Binding rate (%) | | |
|--------------------------------|--------------------|--|--|
| | Milk with standard | Milk with standard and 0.5 mg/mL TF _s | Milk with standard and 1.0 mg/mL TF _s |
| Benzaldehyde | 72.67±2.49 | 48.72±2.51 | 20.93±8.38 |
| (<i>E,E</i>)-2,4-heptadienal | 56.61±4.97 | 67.85±3.06 | 51.89±9.72 |
| Methyl salicylate | 50.53±4.76 | 49.87±4.00 | 34.36±3.57 |
| β -Ionone | 86.12±0.68 | 87.92±0.41 | 78.87±0.27 |
| α -Ionone | 86.90±0.59 | 89.35±0.37 | 80.76±0.88 |
| Linalool | 60.89±4.37 | 76.49±1.64 | 41.40±16.84 |
| Geraniol | 71.48±8.18 | 87.26±1.34 | 54.31±12.43 |