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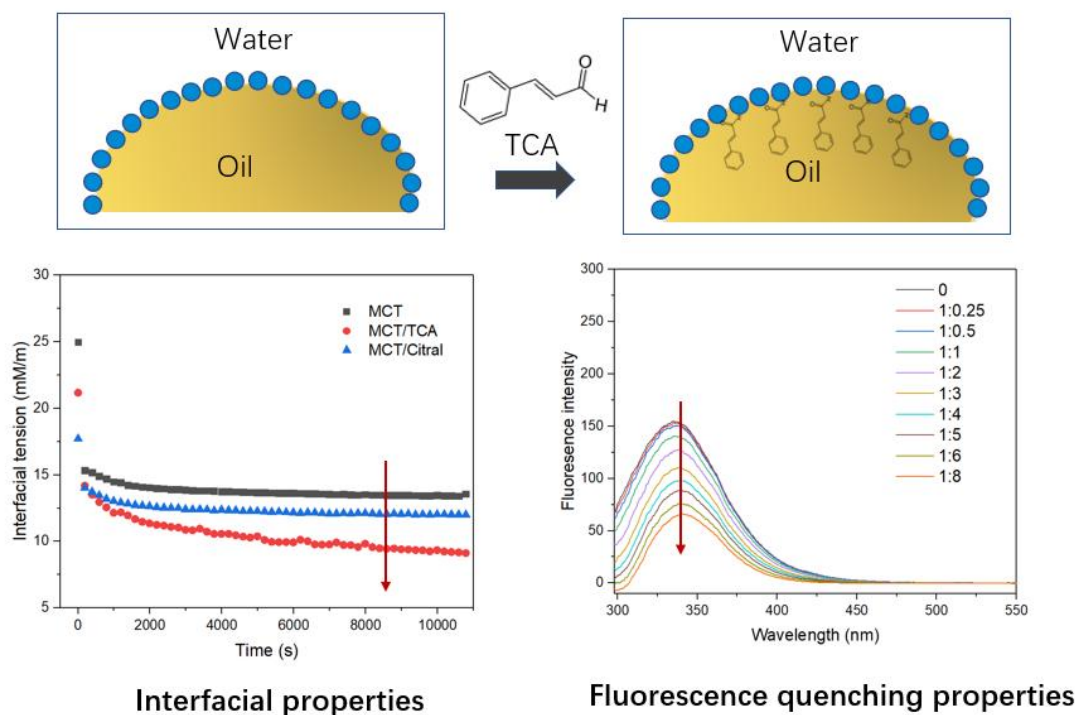
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1 **Effect of trans-cinnamaldehyde or citral on sodium caseinate: Interfacial rheology and**
2 **fluorescence quenching properties**

3

4

5 **Graphical abstract:**



6

7 **Highlights:**

8 ● TCA and citral addition results in lower interfacial tension at O/W interface for CAS
9 solution.

10 ● TCA addition enhances the surface dilatational module of O/W interfacial film.

11 ● The binding between TCA and CAS involves hydrogen bonding with conformation change.

12 ● The binding between TCA and caseinate is spontaneous.

13

14 **Abstract:**

15 *Trans*-Cinnamaldehyde (TCA) and citral are the major components of cinnamon oil and
16 lemongrass oil, respectively, which have a variety of functional properties. The interaction
17 between CAS and these two aldehydes was studied by interfacial properties and fluorescence
18 quenching measurements. The data of protein adsorption in the O/W interface indicated that
19 mixing a small amount of TCA or Citral into the oil phase can both decrease the interfacial tension
20 and the effect of TCA was more prominent. Meanwhile, the use of TCA improved the diffusion
21 rate (K_{diff}) in the initial absorption stage and developed a stronger interface with higher moduli.
22 The fluorescence quenching test showed that TCA has a more obvious quenching effect on CAS
23 than citral. Further kinetic analysis of the binding between CAS and TCA indicated that both
24 dynamic and static quenching occurs. The large binding constant ($1.78 \times 10^5 \text{ M}^{-1}$) at 25 ° C was
25 calculated by Stern-Volmer equation which suggests that TCA has strong affinity for CAS.
26 Moreover, this bonding process is spontaneous, as suggested by negative free energy change. The
27 negative enthalpy and negative entropy change also suggested that the binding is driven by
28 formation of hydrogen with unfavorable conformational change. This work would provide some
29 guidance for by using the binding properties with some nature aldehydes to enhance the interface
30 properties of protein.

31

32 **Keywords:** Sodium caseinate (CAS); *trans*-Cinnamaldehyde (TCA); Citral; Interfacial properties;
33 Fluorescence quenching;

34

35

36 **Introduction:**

37 Many food products are based on emulsions systems (O/W and W/O) (X. Zhang et al., 2021)
38 which require the use of emulsifying agents to stabilize the interface between different phases.
39 Nowadays, food industries prefer to use more nature emulsifiers like proteins rather than chemical
40 synthetic emulsifiers (Felix et al., 2019), since these proteins can providing extra nutritional added
41 values and are more acceptable by consumers. Some proteins such as soy, pea, gelatin, dairy
42 protein have been reported as emulsifiers with good surface activity at the interface, which can
43 adjust the interfacial properties of emulsion systems by partially spreading their hydrophobic
44 groups to the oily phase (Fuller and Vermant, 2012; Ribeiro et al., 2021). During the absorption
45 process, protein molecular general unfold and expose numbers of hydrophobic residues which
46 may physically or chemically interact with other functional compounds, thereby altering their
47 functional performance (Dickinson, 1989). Different active compounds introduced to protein
48 based systems to tailor their solubility, hydrophobicity, dispersibility and surface charge by using
49 of some enzyme or crosslinking agents (Fischer and Windhab, 2011; Glusac and Fishman, 2021;
50 Tann et al., 2001). However, these treatments may introduce a new function group of components
51 which is generally limited in the food section due to toxicity and absence of GRAS status.
52 Therefore, more natural compounds for protein functionalization should be search and
53 investigated.

54 Among flavor compounds, aldehydes have an vital role in flavor development of food
55 products (Wang et al., 2021). It was reported that certain aldehydes are able to interact with
56 various functional residues of amino groups, like ϵ -amino groups of lysine, the sulfhydryl group of
57 cysteines, the imidazole ring of histidines and the phenol group of tyrosines, etc (Azeredo and
58 Waldron, 2016; Habeeb and Hiramoto, 1968). Specific cases include the some aromatic aldehydes
59 such cinnamaldehyde, salicylaldehyde, syringaldehyde, vanillin and anisaldehyde, have been
60 introduced in food and pharmaceutical industries (Banerjee and Chattopadhyay, 2019; Boeve and
61 Joye, 2020). These aromatic aldehydes have gained considerable attention since their various
62 health benefits like anti-inflammatory, antioxidant, antimicrobial agents and anticancer properties
63 (Banerjee and Chattopadhyay, 2019). *Trans*-cinnamaldehyde (3-phenyl-2-propenal, TCA, FEMA
64 2286) is a major component of volatile cinnamon essential oil (Adams et al., 2004). It showed
65 broad-spectrum antimicrobial activity against bacteria, yeast, and molds (Shen et al., 2015). Citral

66 (3,7-dimethyl-2, 6-octadienal, FEMA 2303) is main component of citrus fruit's peel oil which is
67 widely applied as a flavoring additive in foods and beverages (Zhang et al., 2020). As these two
68 active compounds are extremely low solubility in water, it is difficult to prepare food formulations
69 containing these nature aldehydes in solubilized form. There are two main approaches to
70 overcome the insolubility issues: 1) mixing them with carrier oils and then producing it in the
71 form of oil-water systems by some food component emulsifiers since these aldehydes show well
72 solubility in oil; 2) introducing them with a food component may provide a way to deliver nature
73 aldehydes since several reports showed that some aldehydes are properly able to bond with
74 proteins (Asghari et al., 2017; Felix et al., 2019; Weibel and Hansen, 1989). A combination of van
75 der Waals forces, electrostatic forces, hydrogen binding and/or hydrophobic binding may
76 contribute to protein-aldehydes interactions and this should be further analyzed. Several studies
77 reported that aldehydes can bind with amino acid residues of proteins by irreversible covalent
78 binding (Guo et al., 2020), hydrophobic interaction (Wang and Arntfield, 2015) or hydrogen
79 bonding (Tan and Siebert, 2008; Weel et al., 2003), which depend on the specific chemistry and
80 structure of the interacting molecules. Meanwhile, the binding between aldehydes and proteins
81 could be also used in oil in water interface to adjust the interfacial properties. However, the
82 relative reports about the mechanisms of interaction between aromatic aldehydes and protein are
83 still scarce.

84 Sodium caseinate (CAS) is heterogeneous and usually consists of four different polypeptide
85 proteins chains: α (s1)- and α (s2)-caseins, β -casein, and κ -casein (Walstra and Jenness, 1984),
86 which have outstanding amphiphilic properties (Luo et al., 2014). CAS can endow food with
87 certain functionalities, such as water solubility, emulsification, foamability and encapsulation
88 ability. This is really useful because dairy products are considered to be good carriers of
89 biologically active ingredients as they are an integral part of many people's diets. Additionally,
90 CAS can adsorb at O/W interfaces and have ability to fast reduce interfacial tension during
91 emulsification process. Eventually, a densely packed interface can be formed which may exhibit
92 viscoelastic behavior. The characteristics of the interfaces are essential for understanding the
93 stability of O/W emulsion systems. To better understanding the interaction mechanism between
94 protein and nature aldehydes, the first objective the first objective is to investigate the effect of
95 aldehydes on the interfacial and oscillatory dilatational properties of CAS stabilized droplet.

96 Moreover, fluorescence quenching was used to explore the binding properties among selected
97 aldehydes and CAS. The effects of temperature and different concentration of aldehydes on CAS
98 were carried out. In short, understanding the binding properties between nature aldehydes and
99 dairy proteins will contribute food industries to formulate dairy-based products with more health
100 benefit and functional properties.

101

102 **2. Material and methods**

103 **2.1. Materials**

104 *Trans*-cinnamaldehyde (TCA, 99%) and citral (99%) were purchased from Sigma-Aldrich (St
105 Quentin Fallavier, France). Sodium caseinate (CAS) was purchased from Fisher Scientific (United
106 Kingdom) and its protein content measured by the Kjeldahl method was 93.20% (nitrogen
107 conversion factor N = 6.38). A medium chain triacylglycerol oil (MCT) was purchased from
108 Gattefossé (Saint-Priest, France). Analytical grade imidazole (C₃H₄N₂), pure ethanol acetic acid,
109 sodium hydroxide (NaOH), and hydrochloric acid (HCl), were purchased from Sigma-Aldrich
110 Chimie (St Quentin Fallavier, France). All the solutions were prepared in deionized water.

111 **2.2. Interfacial properties**

112 **2.2.1. Sample preparation**

113 The aqueous phase for interfacial measurements was prepared by dispersing CAS powder
114 (0.01 wt.%) into 100 mL of buffer solution (5 mmol/L acetic acid-imidazole, 0.05 wt.% sodium
115 benzoate, pH 7). The protein solution was stirred at room temperature until it was completely
116 dissolved. Oily phases were prepared by mixing TCA or citral (5 wt.%) with MCT under stirring
117 until they are completely homogeneous. Pure MCT was used as control.

118 **2.2.2. Droplet tension measurement**

119 The O/W interfacial properties were measured by a Tracker automatic droplet tensiometer
120 equipped with an oscillating drop accessor (Civrieux d'Azergue, France) at room temperature. The
121 droplet profile was recorded every 0.02s by a connected digital camera. The values of interfacial
122 tension were calculated by the Laplace equation (Castellani et al., 2010). According to the density
123 of the oil phase, the rising drop method were chosen. Three different oil compositions (MCT,
124 TCA/MCT, Citral/MCT). An initial droplet with a volume of 10 µL and a surface area of ~20 mm²
125 was generated at the vertical needle, which was connected to an automatic syringe. The specific

126 low absorbance glass cuvette (8 ml) was filled with the aqueous phase contained 0.01 wt.% CAS.
127 Interfacial tension was performed over a period of 10,800 s to monitor the protein adsorption
128 kinetics. The oscillatory dilatational measurements (five cycles) were carried out at 10%
129 amplitude and at a frequency of 0.1 Hz, which was tested to be within the linear viscoelastic
130 regime. Further dilatational analysis was carried out during the protein adsorption phase, every
131 100 s.

132 **2.3. Binding properties**

133 **2.3.1. Sample preparation**

134 The CAS stock solution (0.048 wt.%, 20 $\mu\text{mol/L}$) was prepared by dispersing CAS powder
135 into 100 mL of pH 7 acetic acid-imidazole buffer solution. The protein solution was stirred at
136 room temperature until it was completely dissolved. The stock solution (160 $\mu\text{mol/L}$) of TCA and
137 citral were prepared by dissolving 2 μL and 2.8 μL , respectively, into 100 mL of 10% ethanol pH
138 7 buffers. Stirring was continued until all of aldehydes were dissolved. Samples for ultraviolet
139 fluorescence quenching test were prepared by mixed required volume of the stock solution of CAS
140 (20 $\mu\text{mol/L}$), aldehydes and pH 7 buffer to the final volume of 4 mL. Therefore, each sample for
141 fluorescence experiments has the fixed concentration (10 $\mu\text{mol/L}$) of CAS and various
142 concentrations of TCA or citral (0, 5, 10, 20, 30, 40, 50, 60 and 80 $\mu\text{mol/L}$). Meanwhile, the
143 highest resulting concentration of ethanol in each sample was 5 wt.% and it have been as control
144 which does not affect the fluorescence intensity of the protein.

145 **2.3.2. Ultraviolet (UV) Spectroscopy**

146 To study the interaction of both aldehydes and CAS, changes in absorption of CAS in the
147 absence and presence of various concentrations of two aldehydes was determined by an UV
148 spectrophotometer (Jenway 3705, Villepinte, France) at room temperature and the wavelength
149 range of 230 - 350 nm. Before each measurement, quartz cuvettes (1 cm path length) were filled
150 with different samples in the dark for 30 min. Sample blank was buffer only.

151 **2.3.3 Intrinsic fluorescence**

152 Intrinsic fluorescence intensity of CAS was carried out in the absence and in the presence of
153 various concentration of TCA or citral at room temperature by multimode microplate reader
154 equipped with a temperature-controlled 96-wells microplate (Thermo Scientific™ Varioskan). The
155 excitation wavelength was set at 280 nm and the emission spectrum was recorded in the

156 wavelength range of 298-550 nm with excitation and emission slit width each of 5 nm. Different
157 samples were placed in the wells as the order of increasing the concentration of aldehydes molar
158 ratio range from 0 to 80 $\mu\text{mol/L}$. The measurements were performed at 25, 30, 35, 40 $^{\circ}\text{C}$,
159 separately. Before each measurement, the mixture of CAS and aldehydes was incubated at room
160 temperature in the dark for 30 minutes. The fluorescence intensity of acetate-imidazole buffer was
161 used as a control.

162 **2.4. Statistical analysis**

163 All measurements were performed at least in triplicate. The results were then reported as
164 averages and standard deviations of these measurements. Statistical analysis was performed by
165 one-way ANOVA using SPSS software (IBM, version 19.0, USA).

166

167 3. Results and discussion

168 3.1. Impact of aldehydes on the interfacial behavior

169 The shape tensiometer has been used for the characterization of complex interfaces since it
170 can provide dynamic information at the O/W interface. In the formation of O/W, hydrophobic
171 groups in CAS will gradually adsorb to the surface of the oil droplets and then after subsequent
172 rearrangements, become an interfacial layer. Fig. 1A showed the plot of interfacial tension as a
173 function of time ($t < 10,800$ s) during the protein adsorption time in the different oily composition.
174 In all three cases, the values of interfacial tension decreased with the time, at least during the
175 initial adsorption period, which is related to different stages of protein adsorption (from protein
176 diffusion to protein penetration, unfolding and rearrangement) (Felix et al., 2017). Pure MCT
177 shows that the equilibrium interfacial tension was relatively high, about 13.56 mN/m, and when
178 mixed with 5 wt.% TCA or citral, the equilibrium interfacial tension was significantly reduced to
179 9.12 and 11.27 mN/m, respectively. A similar result was observed by the study of cinnamaldehyde
180 and whey protein (Felix et al., 2019). one possibility is that these two aldehydes are able to
181 interact with proteins, leading to the additional rearrangements of CAS in the O/W interfaces,
182 thereby resulting in a more densely packed structure, with lower interfacial tension (Dickinson,
183 1999). Interestingly, the image of oil droplets during measurement was recorded in 0, 3 and 15 h
184 as shown in Fig. 1B. After 3 or 15 hours of protein adsorption, the pictures of oil droplet
185 containing TCA showed obvious more turbid than others. This phenomenon can further support
186 the possibility which TCA may have chemically reacted with the adsorbed CAS due to the
187 presence of an aldehyde group in TCA molecules, thereby changing the structure and adsorbed
188 CAS.

189 The change in interfacial tension with absorption time can be fitted with by the equation of
190 Ward and Tordai equation (1) with slightly modification (Ward and Tordai, 1946).

$$191 \pi = 2C_0K_B T \left(\frac{Dt}{3.14} \right)^{1/2} \quad (1)$$

192 Where C_0 is the concentration of continuous phase, K_B is the Boltzmann constant, T is the absolute
193 temperature, and D is the diffusion coefficient. The initial process of adsorption was assumed to
194 be mainly controlled by the protein diffusion (Xiong et al., 2018). The plot of interfacial tension as
195 function of $t^{1/2}$ is linear, and the diffusion rate (K_{diff}) can be calculated by the slope of this plot as

196 shown in [Table 1](#). The highest K_{diff} rate was observed in the TCA/MCT oil phase, while there no
 197 obvious different observed in other two oily phases at the same CAS solution. These results
 198 suggested that for CAS stabilized interfaces, introducing citral into oily phase has only a marginal
 199 effect on protein diffusion. In addition, a small amount of TCA into oil phase can significantly
 200 improve the dispersibility and diffusion properties of CAS at the O/W interfaces. Several studies
 201 have indicated that TCA have the ability to interact with proteins, resulting in the formation of
 202 larger protein species and additional rearrangements in the O/W interface ([Chen et al., 2018, 2017](#)).
 203 After the initial diffusion stage, the adsorption kinetics become visibly slow which was caused by
 204 the energy barrier during the adsorption and rearrangement ([Graham and Phillips, 1979](#)).
 205 Moreover, the rate of penetration and rearrangement of adsorbed proteins at O/W interfaces can be
 206 analyzed by the first-order equation with slightly modification ([Perez et al., 2009; Suttiprasit et al.,](#)
 207 [1992](#)):

$$\ln \left[\frac{\pi_t - \pi_f}{\pi_0 - \pi_f} \right] = -K_i t \quad (2)$$

209 Where π_0 , π_f and π_t are the interfacial tension at the initial, final, and at any time, respectively,
 210 t is adsorption time and K_i is the first-order rate constant. [Fig. 2](#) displayed a typical fitting curve of
 211 $\ln[(\pi_f - \pi_t)/(\pi_f - \pi_0)]$ as a function of the time. All of these curves include in two linear region which
 212 represent the first-order rate constant of penetration (K_p) and molecular reorganization (K_r) at the
 213 O/W interface, respectively. As shown in [Table 1](#), the relative highest K_p value was found in the
 214 pure MCT and TCA/MCT oily phases, which suggested that TCA only have ability to promote the
 215 adsorption speed at the initial processing of protein absorption. Similar K_p values ([Table 1](#)) were
 216 found in pure MCT and TCA/MCT oil phase, which indicates that TCA does not have the ability
 217 to promote protein adsorption during the protein penetration process at O/W interface. This was
 218 probably attributed that TCA interact with adsorbed CAS molecular entanglement by forming a
 219 network structure, and then hindered the protein penetration at the interfacial layer. However, a
 220 relatively low K_p value was found in Citral/MCT oil and the lower R-value was obtained by a
 221 linear fit. This result may cause by the low density of citral (0.888 g/L). The low density of the
 222 mixed oily phase gradually changed the shape of droplets during the protein adsorption. On the
 223 other hand, the highest K_r value was found in the pure MCT oil phase, which be interpreted the
 224 fact that TCA and Citral were introduced into the oily phase and then decrease the polarity of the

225 oily phase (Liao et al., 2021), and lower hydrophobicity of oil phases will reduce the ability to
226 attract emulsifiers.

227 **3.2. Dilatational viscoelastic properties**

228 Measuring the viscoelastic properties of the layers adsorbed at the O/W interface is an
229 effective ways to evaluate the performance of the performance of the emulsion system (Chen et al.,
230 2019). The dilatational elastic dilatational modulus were calculated by the first harmonic of the
231 Fourier transform of the oscillating interfacial tension signal (Bagley and Torvik, 1983). Fig. 3
232 displayed the elastic module of oil-water interface with three different oily phases. After
233 adsorption for 3 hours, the dilatational elastic modulus of all samples was significantly greater
234 than the viscosity modulus (data not shown), indicating that the interface absorbed layer mainly
235 exhibited the elastic behavior. In all cases, the value of elastic module gradually increased as a
236 function of time due to the protein adsorption and the interaction between protein and protein. At
237 the initial process of protein adsorption, the growth of elastic module was obvious and rapid and
238 then it became gradually flattened. This tendency has been previously reported by other proteins,
239 such as soy protein (Wang et al., 2020), bovine serum albumin (Tang and Shen, 2015), and pea
240 protein (Sha et al., 2021). Interesting, the results confirmed that the addition of both aldehydes,
241 especially TCA, can increase the elastic modules and result in a stronger protein network. These
242 findings suggested that the interaction between protein and aldehydes had a vital influence on the
243 protein adsorption at the O/W interface.

244 **3.3. Ultraviolet Spectroscopy**

245 UV spectroscopy is one of the generally used techniques for the illumination of the binding
246 mode of small molecules to proteins, by measuring the changes in absorption and shift in the
247 wavelength (Alam et al., 2018). The effect of various TCA and Citral concentrations (0 -80
248 $\mu\text{mol/L}$) on the CAS solution (10 $\mu\text{mol/L}$) was shown in Fig. 4. Pure TCA and Citral (40 $\mu\text{mol/L}$
249 of, buffer) were used as the control. Fig. 4A showed that the mixture of CAS and TCA exhibited a
250 maximum absorbance in the range of 280-290 nm and a distinct red shift was observed.
251 Meanwhile, the intensity of the maximum absorbance was significantly improved with the
252 increasing TCA concentration, indicating that there was interaction between TCA and CAS.
253 Moreover, the adsorption peak at 280 nm of the mixture suggested that high concentrated TCA
254 may cause the tryptophan, tyrosine and phenylalanine residues become more accessible and

255 therefore causes conformational changes in the protein (Sudha et al., 2016). As shown in Fig. 4B,
256 pure citral exhibited a maximum absorbance around 245 nm and the mixture of CAS and TCA
257 exhibited a similar maximum intensity around 280 nm, which suggesting there is no obvious
258 interaction between CAS and citral.

259 3.4. Fluorescence spectra

260 The fluorescence spectra of CAS with the various concentrations of TCA and Citral at 25 °C
261 are shown in Fig. 5. The CAS solution with the presence and absence of aldehydes possessed
262 different fluorescence intensities which indicated the changes of environmental conditions. As
263 several studies reported (Acharya et al., 2013), pure CAS solution exhibited an obvious emission
264 band around 350 nm when the extraction band was 280 nm. After mixing with TCA (Fig. 5A), the
265 fluorescence intensity of the maximum emission band decreased, which further indicated the
266 occurrence of fluorescence quenching and the formation of complexes by TCA binding to
267 aromatic residues of CAS. This fluorescence quenching have been supported by many previous
268 studies (Mohammadian et al., 2019; Shi et al., 2015; Wu et al., 2021). With the subsequent
269 increase in the TCA concentration, the maximum intensity further decreases with a gradual red
270 shift, which may cause by the presence of binding sites near the fluorophores in the protein
271 (Soltanabadi et al., 2018). However, as shown in Fig. 5B, the fluorescence intensity of CAS and
272 citral only shows a very weak quenching phenomenon, which suggested CAS molecular could not
273 provide a suitable environmental structure to bind citral molecular.

274 The quenching of CAS induced by TCA can be further analyzed by the Stern-Volmer
275 equation (3), which can be dynamic, a collision between the fluorophore and quencher, or static,
276 the formation of a ground-state complex between the fluorophore and quencher (Lakowicz, 2013).

$$277 \quad \frac{F_0}{F} = 1 + k_q \tau_0 [Q] = 1 + K_{SV} \quad (3)$$

278 Where F_0 and F are the fluorescence intensities at 280 nm excitation wavelength before and after
279 mixing with the quencher, respectively; k_q is the bimolecular quenching constant, τ_0 is the average
280 lifetime of the fluorophore in the absence of quencher; $[Q]$ is the concentration of the quencher,
281 and K_{sv} is the Stern-Volmer quenching constant which can be calculated by linear regression of a
282 plot of F_0/F against $[Q]$. The curve of F_0/F as a function of quencher concentration $[Q]$ at different
283 temperatures was shown as Fig. 6A. It is worth to note that, at high TCA concentration, an upward

284 deviation from linearity can be observed, which suggested that both dynamic and static quenching
285 may occur in the fluorescence quenching mechanism. However, this equation is still not enough to
286 distinguish the dynamic quenching from static (Acharya et al., 2013). An effective way to
287 distinguish between dynamic quenching and static quenching can be to observe their bonding
288 performance at different temperatures. The temperature increase can increase the quencher
289 diffusion constant, which is usually accompanied by an increase in collisional quenching. For
290 static quenching, the temperature have different effects (Acharya et al., 2013). Typically, this
291 process will result in a decrease in the binding constant of the fluorophore quencher, and if the
292 complexation reaction is exothermic, it will result in a decrease in the quenching of the static
293 quencher. The modified Stern-Volmer equation (4) (Lakowicz and Weber, 1973) can be used to
294 analysis when both static and dynamic quenching occur.

$$295 \quad \frac{F_0}{F_0-F} = \frac{1}{f} + \frac{1}{f} \frac{1}{K_{SV}} \frac{1}{[Q]} \quad (4)$$

296 Where f is the available binding sites. When f is 1, the equation become the simple quenching
297 process equation (3). The curve of $F_0/(F_0-F)$ as a function of quencher concentration $[Q]^{-1}$ at
298 different temperatures was shown as Fig. 6B. At 25 °C, the quenching of CAS by TCA, K_{sv} is 2.25
299 $\times 10^{-4} \text{ M}^{-1}$. The fraction of the binding sites available for complexation was 1.63 higher than 1.0,
300 which was probably attributed the occurrence of both dynamic and static quenching
301 simultaneously. In addition, more binding sites could also be related with CAS which are a
302 mixture of different casein protein, such as α_{s1} , α_{s2} , β and κ - casein and there caseins differ in the
303 number of tryptophan residues and their location in the hydrophobic regions (Fu et al., 2014;
304 Yazdi and Corredig, 2012).

305 The fluorescence lifetime of tryptophan has been reported around 3 ns (Lakowicz and Weber,
306 1973). The K_q , calculated by the equation (3), is $7.5 \times 10^{12} \text{ M}^{-1}$ per second at 25 °C, which is
307 highly greater than the maximum dynamic quenching constant ($\sim 10 \times 10^{10} \text{ M}^{-1} \text{ s}^{-1}$). This indicated
308 that there was a specific complex formation between CAS and TCA. For further analysis of
309 quenching interaction, the binding constant and binding sites can be calculated by following
310 modified Stern-Volmer equation (5).

$$311 \quad \log \left[\frac{F_0-F}{F} \right] = \log K + n \log [Q] \quad (5)$$

312 Fig. 6C exhibited the curves of $\log [(F_0-F)/F]$ against $\log [TCA]$ for the quenching of CAS

313 intrinsic fluorescence by TCA. [Table 2](#) displayed the quenching parameter K (binding constant)
314 and n (binding sites) at different temperatures. The large values of K were obtained at 25 and
315 30 °C which indicated the binding of TCA with CAS is highly favorable. As increasing
316 temperatures, the binding constants gradually decreased which suggested that the complexation
317 becomes weak at higher temperatures. Similar results was also found in the study of resveratrol
318 and whey proteins by fluorescence spectroscopy ([Hemar et al., 2011](#)). The n values for binding of
319 CAS and TCA were close to 1.0 for different temperatures.

320 To a better understanding of the thermodynamic of the interaction between CAS and TCA,
321 Van't Hoff equation (6) was followed to calculate the change of enthalpy ΔH and entropy ΔS .

$$322 \quad \ln K = -\frac{\Delta H}{RT} + \frac{\Delta S}{R} \quad (6)$$

323 Where K is binding constant, R is the gas constant (8.314 J mol⁻¹k⁻¹) and T is the absolute
324 temperature (K). Thereby, thermodynamic parameters ΔH and ΔS can be calculated by the slope
325 and intercept of the curve of $\ln K$ as a function of $1/T$. [Fig. 7](#) showed the current Vant's Hoff fitting
326 curve and it gives $\Delta H = -55.76$ kJ mol⁻¹, $\Delta S = -86.05$ J mol⁻¹. The free energy change (ΔG) can be
327 then calculated from the equation (7) as follow:

$$328 \quad \Delta G = \Delta H - T\Delta S \quad (7)$$

329 [Table 2](#) summarized the thermodynamic parameters of binding between CAS and TCA. ΔG values
330 at different temperatures are all negative, indicating the binding process of TCA and CAS is
331 spontaneous and exothermic. The negative ΔH and ΔS values suggested that binding would be
332 mainly driven by hydrogen bonds, which would promote a more ordered configuration of the
333 solvent molecules around the complexes. This similar interaction was also found for other
334 protein–small molecular combinations, such as zein proteins–resveratrol ([Joye et al., 2015](#)),
335 crosslinked caseinate- quercetin, caseinate- kaempferol ([Y.-J. Zhang et al., 2021](#)) and bovine
336 serum albumin- theaflavin-3,3'-digallate ([Yu et al., 2022](#)). Moreover, the high absolute value of
337 ΔH even indicates that a covalent bond may be formed between CAS and TCA. Other
338 complementary technologies are needed in the future to fully explore the exact type and strength
339 of interaction between CAS and TCA. Nevertheless, the present work showed that TCA, a
340 common flavoring in food, may be used as an interesting ingredient to improve the surface
341 rheological properties of protein-based interfaces.

343 **4. Conclusion**

344 The interaction between aldehydes and CAS was investigated in the current study. Both aldehydes,
345 especially TCA, have pronounced abilities to decrease the interfacial tension at O/W interface
346 during adsorption process. Small amount of TCA introduced into oil phase can significantly
347 improve the dispersibility and diffusion properties of CAS at the O/W interfaces. The results of
348 dilatational measurements indicated the use of TCA into oily phase resulted in the development of
349 stronger and more elastic interfaces. Furthermore, the fluorescence quenching test proved that
350 TCA have more obvious quenching effect on CAS than citral. The large binding constants
351 ($1.78 \times 10^5 \text{ M}^{-1}$) was calculated by Stern-Volmer equation which suggest that TCA has strong
352 affinity with CAS. The negative free energy change for the binding between TCA and CAS
353 indicated that complexation process is spontaneous. The negative enthalpy and negative entropy
354 change suggested that the binding is mainly driven by formation of hydrogen. Entropy is
355 unfavorable suggesting there is a loss of conformational freedom associated with binding. In
356 summary, these nature aldehydes, could be a useful ingredient to improve the functionality of food
357 protein-based products.

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359

360

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363 **CONFLICTS OF INTEREST**

364 The author declares that there is no conflict of interest that could be perceived as prejudicing the
365 impartiality of the research reported

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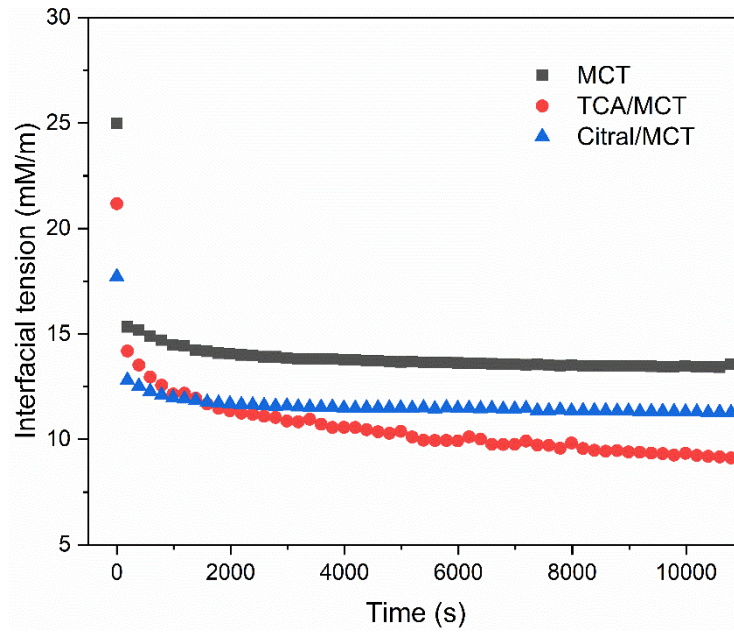
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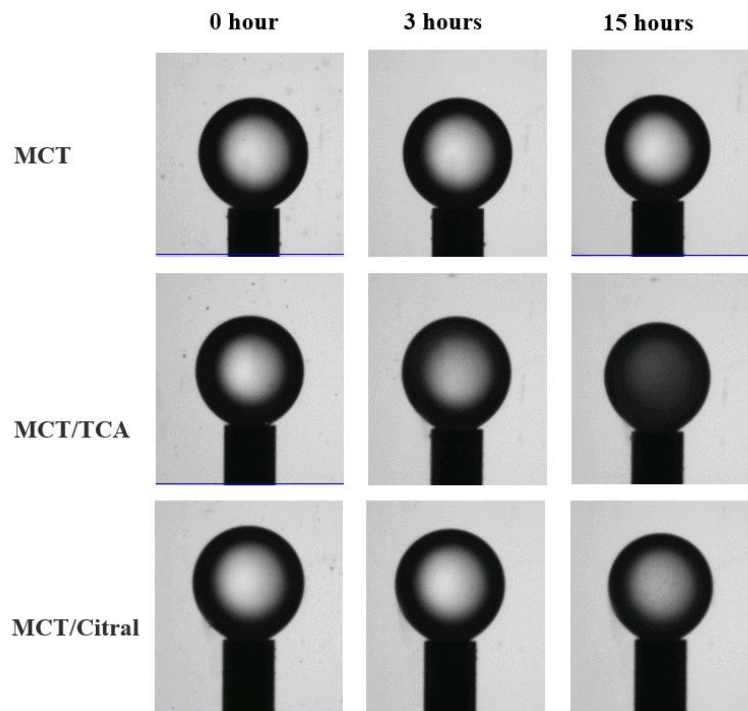
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(A)



(B)

Fig. 1. (A) Interfacial tension over protein adsorption at O/W interface with or without TCA or Citral for 0.01% CAS solution at pH 7; (B) Visual image of oil droplets during measurement at different time.

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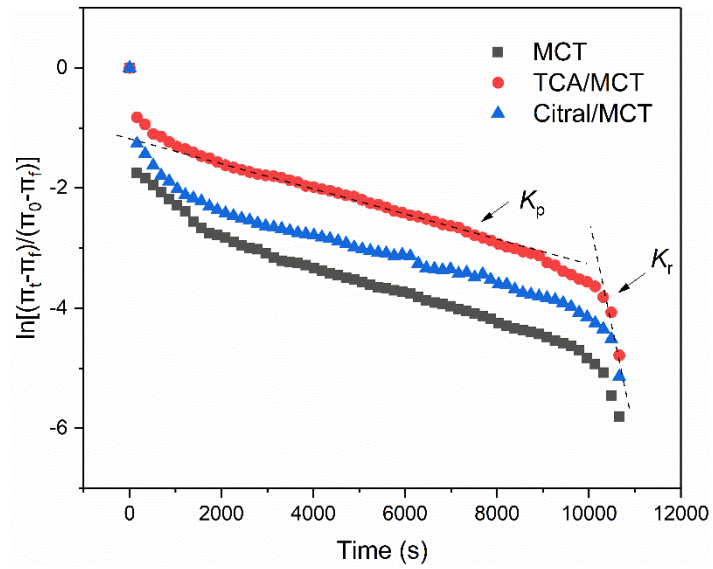
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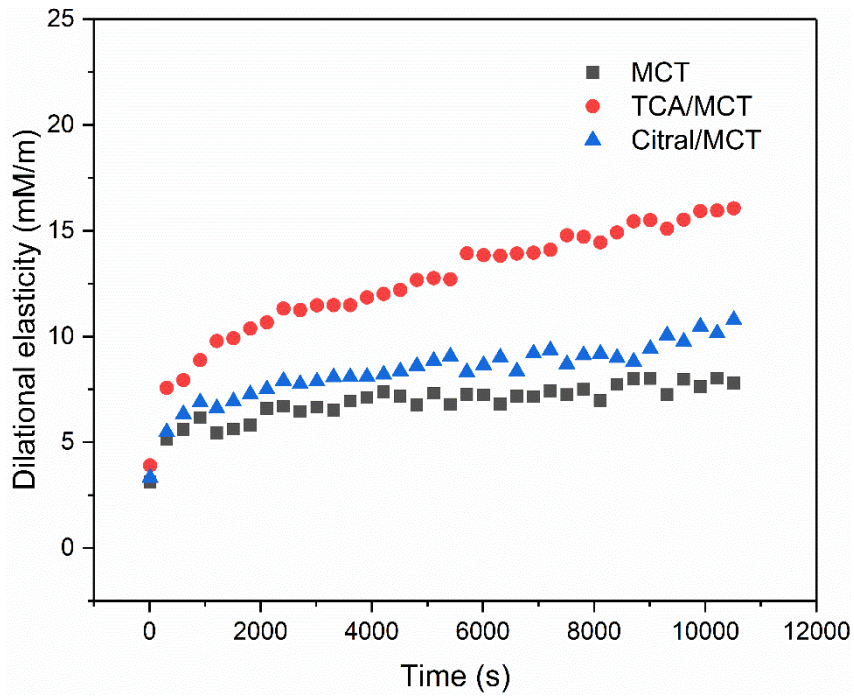
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531 **Fig. 2.** The profile of the molecular penetration and configurational rearrangement steps over
 532 protein adsorption at the O/W interface with and without TCA or Citral for 0.01% CAS
 533 solution. K_p and K_r represents the first-order rate constants of penetration and rearrangement,
 534 respectively.

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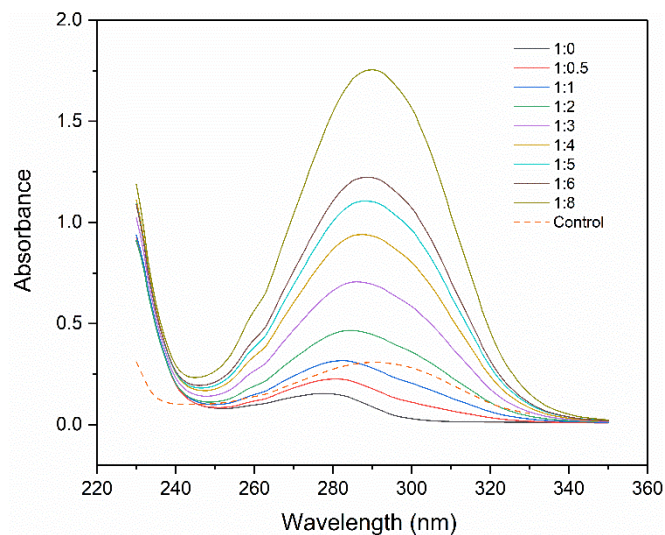
Fig. 3. Dilatational elasticity over protein adsorption at O/W interface with and without TCA or

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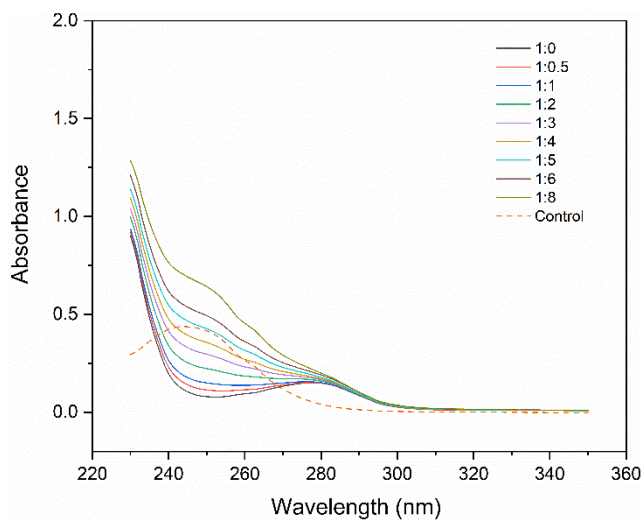
Citral for 0.01% CAS solution.

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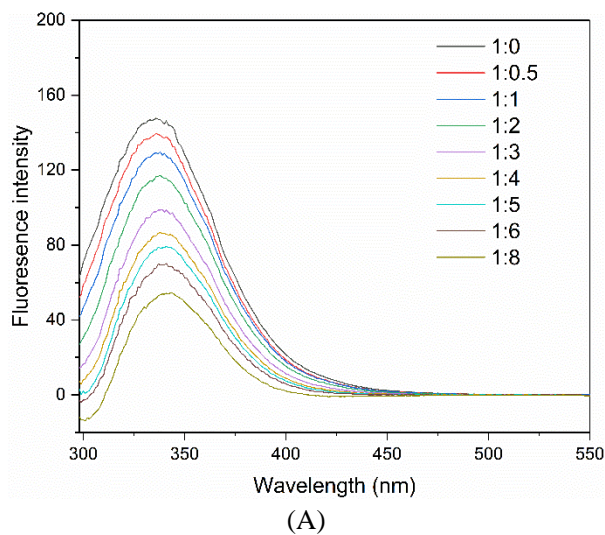
(A)



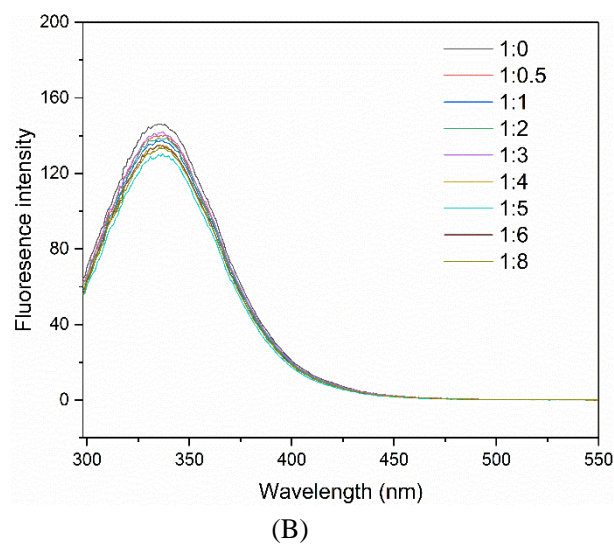
(B)

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Fig. 4. Absorption spectra of 10 μmol/L CAS solution in the presence of increasing (A) TCA or (B) citral concentrations (0 - 80 μmol/L) at 25 °C.



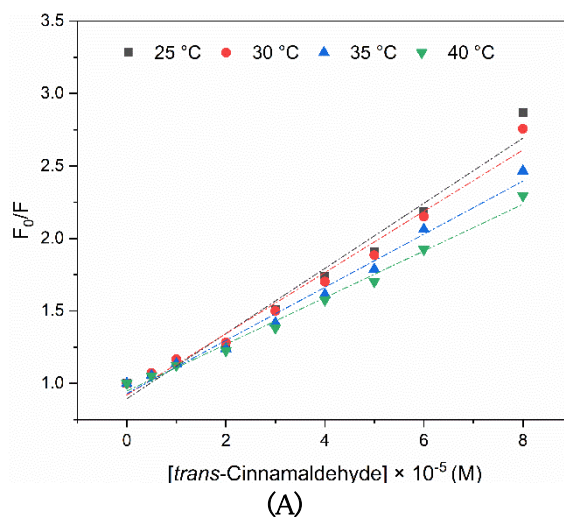
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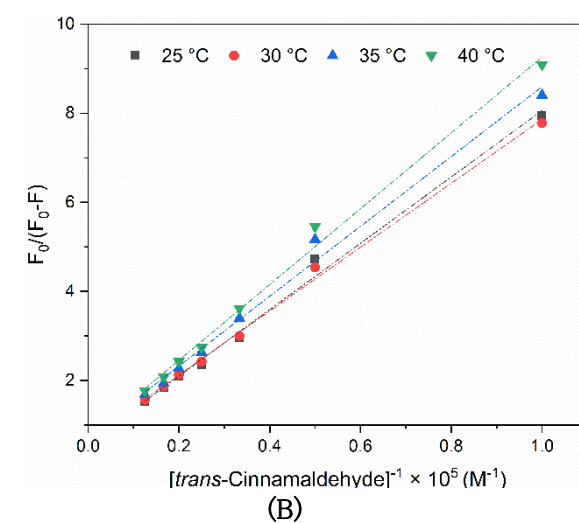
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563 **Fig. 5.** Fluorescence spectra of 10 $\mu\text{mol/L}$ CAS solution at the excitation wavelength of 280 nm in
 564 the presence of increasing (A) TCA or (B) citral concentrations (0 - 80 $\mu\text{mol/L}$) at 25 $^{\circ}\text{C}$.

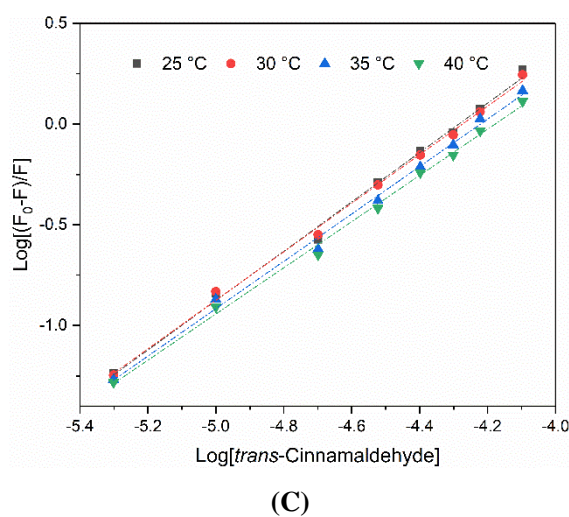
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569 **Fig. 6.** (A) Stern–Volmer plot of F_0/F as a function of the concentration of TCA at different
 570 temperatures; (B) Plot of $(F_0/(F_0-F))$ as a function of the reciprocal of concentration of TCA at
 571 different temperatures; (C) Plot of $\text{Log} [(F_0 - F)/F]$ as a function of log of the concentration of
 572 TCA at different temperatures; F_0 and F represent the fluorescence intensity in the absence and

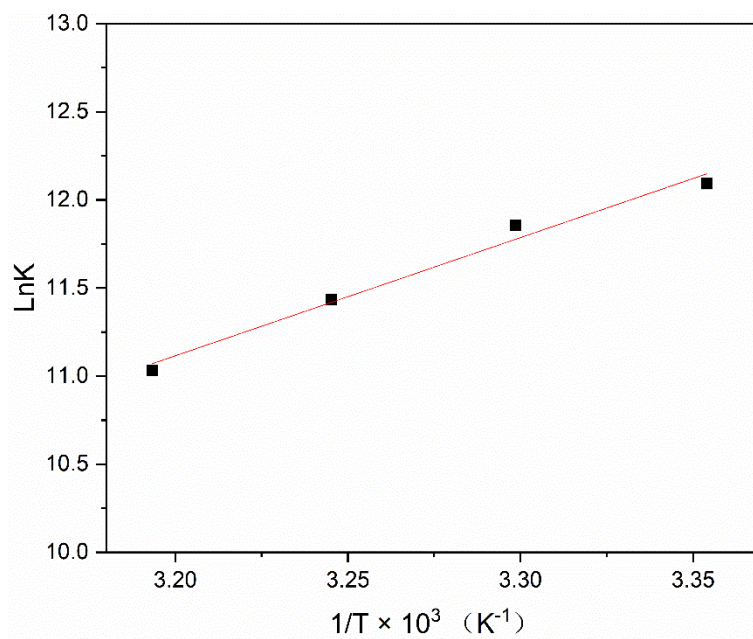
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presence of TCA, respectively.

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578 **Fig. 7.** The Van't Hoff plot of ln K as a function of 1/T (K) for the binding of TCA with CAS
579 solution.

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582 **Table 1.** the apparent diffusion rate (K_{diff}), constants of penetration and structural rearrangement at
 583 the interface (K_p and K_r), and interfacial pressure at the end of adsorption (π_{10800}) for different oily
 584 phase in 0.01% CAS solution.
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Samples	K_{diff} (mN/m/s ^{1/2})	$K_p \times 10^4$	$K_r \times 10^4$	π_{10800} (mN/m)
MCT	-0.090 ± 0.016	-2.151 ± 0.005	-8.543 ± 0.005	13.56
TCA/MCT	-0.122 ± 0.006	-2.178 ± 0.001	-7.046 ± 0.009	9.12
Citral/MCT	-0.081 ± 0.010	-1.820 ± 0.002	-5.626 ± 0.002	11.27

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Table 2. Variation of binding constant K and the number of binding site n as a function of

T (°C)	K	n	r^2	ΔH (kJ mol ⁻¹)	ΔS (J mol ⁻¹ K ⁻¹)	ΔG (kJ mol ⁻¹)
25	1.782×10^5	1.225	0.996			-30.1051
30	1.406×10^5	1.204	0.997			-29.6748
35	0.925×10^5	1.177	0.996	-55.76	-86.05	-29.2446
40	0.618×10^5	1.147	0.997			-28.8143

597 temperature and thermodynamic parameters of binding calculated using Van't Hoff equation.
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 599