



# Effect of xanthan gum on the release of strawberry flavor in formulated soy beverage



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## ABSTRACT

The effects of xanthan gum on the release of strawberry flavor compounds in formulated soy protein isolate (SPI) beverage were investigated by headspace gas chromatography (GC). Seven strawberry flavor compounds (limonene, ethyl hexanoate, (Z)-3-hexenyl acetate, ethyl 2-methylbutanoate, ethyl butanoate, (Z)-3-hexen-1-ol and diacetyl) could be detected by GC and hence analyzed the gas-matrix partition coefficients (*K*). The release of flavor compounds was restrained in SPI and/or xanthan gum solution. The retention of (Z)-3-hexen-1-ol, limonene and diacetyl significantly changed ( $p < 0.05$ ) with increasing xanthan gum concentrations. Presence of any other esters led to suppression of the release of ester compounds in water and SPI solution. The less-volatiles ( $\gamma$ -decalactone, methyl cinnamate, hexanoic acid, 2-methyl butyric acid and furaneol) accelerated the release of ester compounds to some extent in different matrices. The above results demonstrated that presence of SPI and xanthan gum could bring about an imbalance in the strawberry flavor.

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## 1. Introduction

Acceptability of foods by consumers mainly relies on their sensory attributes, among which flavor perception plays an important role. Flavor compounds can be naturally present in foods or can be added to balance. Thus, the perception may change both due to

changes in volatility of the flavor compounds or a small modification of a food matrix, which consequently could affect the overall flavor profiles (Heilig, Cetin, Erpenbach, Hohn, & Hinrichs, 2011).

Soy proteins have become popular among consumers owing to their abundant supply, relatively low cost and nutritive value (Arora & Damodaran, 2010). However, the consumption of soy foods is still limited in mainstream food applications, due to the presence of undesirable beany or grassy off-flavors (Endo, Ohno, Tanji, Shimada, & Kaneko, 2005). Furthermore, flavor compounds added to a soy food product may interact with soy protein or other ingredients (Evageliou & Patsiakou, 2014; Moon & Li-Chan, 2007),

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resulting in an imbalance in the flavor profile. Hydrocolloids, which can modify the rate and intensity of flavor release through diffusion, caging in by gel effect, trapping in micro-regions, molecular interactions and molecular inclusion (Bylaite, Adler-Nissen, & Meyer, 2005), are among the most used additives in industrially manufactured beverage foods. Previous studies have shown the influence of hydrocolloids on the release of flavor compounds in different model systems. For instance, binding studies with acacia gum (Savary, Hucher, Petibon, & Grisel, 2014), gellan, pectin (Evageliou, Papastamopoulou, Frantzeskaki, & Christodoulidou, 2015) or gelatine (Zafeiropoulou, Evageliou, Gardeli, Yanniotis, & Komaitis, 2012) have been reported. Kühn, Delahunty, Considine, and Singh (2009) researched the influence of sodium carboxymethylcellulose (CMC) on the interaction between milk protein and 2-nonanone. The effect of hydrocolloids on flavor compounds in complex systems such as yoghurt or dairy were also studied (Decourcelle, Lubbers, Vallet, Rondeau, & Guichard, 2004; Lubbers, Decourcelle, Martinez, Guichard, & Tromelin, 2007; Philippe et al., 2003). However, it is less clear how hydrocolloids influence the perception when present in a soy protein beverage.

On the other hand, most published papers to date have selected single volatile model compounds or homologous series of aldehydes, ketones or alcohols (Damodaran & Kinsella, 1980; Kühn, Zhu, Considine, & Singh, 2007; Wang & Arntfield, 2014). Despite the great amount of research dealing with the actual flavoring system (Boland, Delahunty, & Vanruth, 2006; Decourcelle et al., 2004; Heilig et al., 2011; Martuscelli, Savary, Pittia, & Cayot, 2008; Moon & Li-Chan, 2007; Vidrih, Zlatic, & Hribar, 2009), relatively limited studies have been performed on the interaction within non-homologues on the release of flavor compounds. For instance, Wang and Arntfield (2015) researched the competitive binding between heterologous 2-hexanone and hexanal. Thus, numerous studies on the release of flavor compounds in model systems have been published, and they promoted the understanding of the interactions of flavor compounds with food ingredients. However, due to the lack of specific information about imbalance of flavor profile in a complicated flavoring system or real food system, the commercial value of these reports could be limited.

For an efficient adjustment or balance of flavor in processed foods or beverages with soy protein ingredients, it is important to understand not only how the individual food ingredients like soy proteins or xanthan gum interact with specific flavor compound in model systems, but also how various coexistence complex flavor compounds would affect flavor release in different food matrices. The aim of this study was to better understand the behavior of strawberry flavor compounds in model systems. The effect of xanthan gum addition on the interaction between strawberry flavor compounds and SPI was investigated. The simplified method, namely phase ratio variation (PRV) method, was used to explore the partition coefficients of the flavor compounds in different matrices. The results can pave the way for further research to elucidate strategies maximizing perception of strawberry flavor in soy beverage products.

## 2. Materials and methods

### 2.1. Materials

The SPI was isolated using the process described by Feng and Xiong (2003). Xanthan gum was kindly donated by Danisco Co. (Copenhagen, Denmark). The strawberry flavor composition used for this study was based on a previous research (Heilig et al., 2011). All flavor compounds were obtained from J&K Scientific Ltd (Beijing, PR China). Their composition and content in strawberry flavoring is reported in Table 1. The purity of flavor

compounds was evaluated by GC-FID (>99%). Analytical grade reagents, sodium phosphate monobasic ( $\text{NaH}_2\text{PO}_4$ ), sodium phosphate dibasic ( $\text{Na}_2\text{HPO}_4$ ), propylene glycol and sodium azide, were purchased from Sinopharm Chemical Reagent Co., Ltd. (Suzhou, PR China). All solutions were prepared using distilled water.

### 2.2. The preparation of flavor stock solutions

Stock solutions of each flavor compound were prepared in propylene glycol. The final concentration of individual flavor compound in matrices were as follows: limonene (0.882 mmol/L), ethyl hexanoate (0.417 mmol/L),  $\gamma$ -decalactone (0.176 mmol/L), methyl cinnamate (0.185 mmol/L), (*z*)-3-hexenyl acetate (0.423 mmol/L), ethyl 2-methylbutanoate (0.769 mmol/L), hexanoic acid (1.552 mmol/L), ethyl butanoate (0.690 mmol/L), (*Z*)-3-hexen-1-ol (1.000 mmol/L), 2-methylbutyric acid (1.765 mmol/L), furaneol (0.211 mmol/L), diacetyl (1.395 mmol/L). The concentration of flavor compounds in different type of mixtures was also the same for each individual flavor compound. The stock solutions were stored at 4 °C for 3 months.

### 2.3. Sample preparation

Solutions of SPI (2% w/w) and xanthan gum (0.1% w/w) were dispersed in 0.05 M phosphate buffer (pH 7.0) and stirred for at least 4 h. The xanthan gum solution was warmed to 50 °C during the magnetic stirring to increase solubility. Solutions of SPI (2% w/w) and xanthan gum were dispersed in phosphate buffer as described above. There were two homogenization steps: firstly, a coarse solution was prepared using IKA Ultra-Turrax T18 homogenizer (Daig-ger Scientific Inc., Chicago, IL) at a speed of 13500 rpm for 1 min. Secondly, high pressure homogenization was carried out with Nano Homogenizer (ATS Engineering Inc., Brampton, Canada) at 20 MPa. Sodium azide (0.02% w/w) was added to all solutions to inhibit microbial growth. The amount of sodium azide used in this study was lower than the level which may interfere with the binding of flavor compounds to the matrix. The solutions were kept at 4 °C for no more than one week. Flavor compounds were added to the prepared solution including water, SPI (2%, w/w), xanthan gum (0.1%, w/w) and the mixture of SPI and xanthan gum (SPI: 2%, w/w; xanthan gum: 0.05% and 0.1%, w/w).

Different volumes of the flavored samples were transferred into 22-mL headspace vials and immediately sealed using PTFE septa in metallic caps (Shimadzu, Kyoto, Japan). These were placed in an incubator at 37 °C. Preliminary experiments of headspace analysis at different equilibrium times were used to ensure that the analysis for each sample was performed at equilibrium. A time of 48 h was sufficient to reach equilibrium for each matrix and flavor compound.

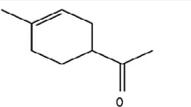
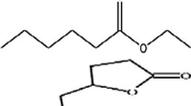
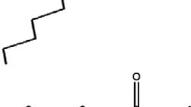
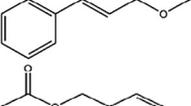
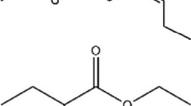
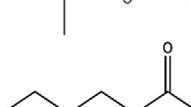
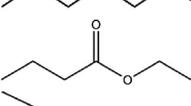
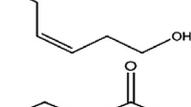
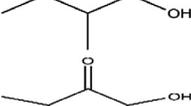
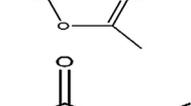
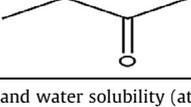
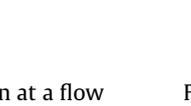
### 2.4. Determination of the gas-matrix partition coefficient

#### 2.4.1. Static headspace gas chromatographic analysis

Static headspace gas chromatographic (SH-GC) analysis was performed using a gas chromatograph (2010 Ultra; Shimadzu, Kyoto, Japan) equipped with a flame-ionization detector and an automatic headspace sampler. Samples were kept at 37 °C for 40 min without stirring in the automated headspace unit until connected to the GC. Vial pressurization time was 2 min and sample injection time was 1 min. The gas used to pressurize the samples was nitrogen.

A capillary DB-1 column was employed (length: 60.0 m, internal diameter: 0.32 mm, film thickness: 0.50  $\mu\text{m}$ ; J&W Scientific, Folsom, CA). The injector port temperature was 200 °C, and the detector temperature was 260 °C. The conditions for gas chromatography were as follows: the oven temperature increased from

**Table 1**  
Physicochemical properties of the strawberry flavor mixture.

Flavor compounds (chemical formula)	CAS number	Molecular weight <sup>a</sup> (g/mol)	Molecular formula <sup>a</sup>	Log <i>P</i> <sup>a</sup>	Vapor pressure <sup>a</sup> (mm Hg)	Boiling point <sup>a</sup> (°C)	Water solubility <sup>a</sup> (g/L)	% (w/w) in strawberry flavoring
Limonene (C <sub>10</sub> H <sub>16</sub> )	138-86-3	136		+4.38	1.45	168	0.005	1.20
Ethyl hexanoate (C <sub>8</sub> H <sub>16</sub> O <sub>2</sub> )	123-66-0	144		+2.83	1.64	170	0.629	0.60
γ-Decalactone (C <sub>10</sub> H <sub>18</sub> O <sub>2</sub> )	706-14-9	170		+2.72	0.005	282	0.292	0.30
Methyl cinnamate (C <sub>10</sub> H <sub>10</sub> O <sub>2</sub> )	1754-62-7	162		+2.62	0.012	240	0.387	0.30
(Z)-3-hexenyl acetate (C <sub>8</sub> H <sub>14</sub> O <sub>2</sub> )	3681-71-8	142		+2.61	1.14	177	0.481	0.60
Ethyl 2-methylbutanoate (C <sub>7</sub> H <sub>14</sub> O <sub>2</sub> )	7452-79-1	130		+2.26	8.03	135	1.07	1.00
Hexanoic acid (C <sub>6</sub> H <sub>12</sub> O <sub>2</sub> )	142-62-1	116		+1.92	0.278	208	10.3	1.80
Ethyl butanoate (C <sub>6</sub> H <sub>12</sub> O <sub>2</sub> )	105-54-4	116		+1.85	14.6	126	4.9	0.80
(Z)-3-hexen-1-ol (C <sub>6</sub> H <sub>12</sub> O)	928-96-1	100		+1.61	0.937	166	16	1.00
2-methylbutyric acid (C <sub>5</sub> H <sub>10</sub> O <sub>2</sub> )	116-53-0	102		+1.18	1.12	175	45	1.80
Furaneol (C <sub>6</sub> H <sub>8</sub> O <sub>3</sub> )	3658-77-3	128		+0.82	0.001	259	18.5	0.27
diacetyl (C <sub>4</sub> H <sub>6</sub> O <sub>2</sub> )	431-03-8	86		-1.34	70.2	88	200	1.20

<sup>a</sup> Molecular formula, Log *P*, vapor pressure (at 25 °C), boiling point and water solubility (at 25 °C): when no indication, from *EPI* suite 4.1 calculation.

60 °C at 10 °C/min to 180 °C. The carrier gas was nitrogen at a flow rate of 1.5 mL/min. For the FID detector, air and hydrogen flow rates were 400 and 40 mL/min, respectively. Data acquisition was achieved using GC Solutions Software (Shimadzu, Kyoto, Japan).

#### 2.4.2. Analysis of headspace gas chromatographic data

The gas-matrix partition coefficient of the flavor compounds can be measured using PRV method, which was based on the influence of the volume of the sample on the concentration of flavor compound in the headspace (Ayed et al., 2014; Lafarge et al., 2014; Tromelin et al., 2012; Van Durme & Werbrouck, 2015).

$\beta$  is the ratio of gas and matrix phase volume. By plotting the inverse of the peak area (1/*A*) against  $\beta$ , a linear zone where *a* and *b* are the slope and intercept was obtained.

$$\frac{1}{A} = a\beta + b \quad (1)$$

From Eq. (1), the partition coefficient can be determined as  $K = a/b$ .

Moreover, the percentage of retention (*R*) can also be calculated:

$$R(\%) = \left(1 - \frac{K_2}{K_1}\right) \times 100 \quad (2)$$

$K_1$  and  $K_2$  represent the partition coefficients for water and matrices respectively. A positive percentage value indicates a flavor compound retained by the matrix, and negative if it is released.

Increasing volumes (0.05, 0.1, 0.2, 0.5 and 1 mL) of the flavored matrices were placed into headspace vials (22 mL). Thus, each vial represented a gas-matrix phase ratio  $\beta$  of 439, 219, 109, 43 and 21 respectively. The PRV method had a linear correlation coefficient ( $r^2$ ) higher than 0.96 in all cases.

The ratio between the partition coefficients of different type of flavor compounds mixtures and single flavor compound was defined. When the ratio value is >1, the release of compound is promoted by other flavor compounds whereas when the value is smaller than 1, it is restrained.

### 2.5. Statistical analysis

All data of the triplicate measurements for this study were adopted for analysis of variance (ANOVA) to determine significant differences among the samples with regard to flavor compounds partition coefficients and retention. The significance of such differences between mean values was determined using Duncan's test ( $p < 0.05$ ). ANOVA and Duncan's multiple range test were performed with SPSS (version 19.0, SPSS, Chicago, IL).

## 3. Results and discussion

### 3.1. Performance of the headspace gas chromatography

Under the experimental conditions of this research such as the preparation conditions, compounds concentration and temperature, only seven (limonene, ethyl hexanoate, (Z)-3-hexenyl acetate, ethyl 2-methylbutanoate, ethyl butanoate, (Z)-3-hexen-1-ol and diacetyl) of 12 strawberry flavor compounds were detected and hence analyzed for their gas-matrix partition coefficients. The five undetected flavor compounds ( $\gamma$ -decalactone, methyl cinnamate, hexanoic acid, 2-methylbutyric acid and furaneol) could be attributed to a combination of low volatility, high boiling point as well as their relative low concentration in the matrix (González-Tomás, Bayarri, Taylor, & Costell, 2007; Heilig et al., 2011; Martuscelli et al., 2008). Additionally, the mixtures of strawberry flavor compounds could be defined by their detection by headspace GC; the five undetected flavor compounds were defined as the less-volatiles, while the other seven strawberry flavor compounds were the volatiles.

### 3.2. Partition coefficients from aqueous solutions

The gas-matrix partition coefficients under equilibrium conditions were calculated using the PRV method to evaluate the interactions between flavor compounds and system components. Generally, the partition coefficients of flavor compounds (Table 2) in SPI, xanthan gum and the mixture of SPI and xanthan gum solutions highly depend on the hydrophobicity and vapor pressure of these compounds (Table 1). The more hydrophobic compounds had higher  $K$  value, showing that the more hydrophobic compounds were more volatile. The least hydrophobic compound (diacetyl with  $\log P = -1.34$ ) was retained to a larger extent due to its greater affinity for water. It was worth noting that ethyl 2-methylbutanoate had a bigger  $K$  value than limonene in water

and the mixture of SPI and xanthan gum. Table 1 showed that ethyl 2-methylbutanoate possessed higher vapor pressure in water compared to limonene, which could explain the behavior of ethyl 2-methylbutanoate.

In the SPI solution, all the studied flavor compounds were better retained than in water (Table 3), illustrating that interaction between SPI and flavor compounds has occurred. Notably, ethyl hexanoate (C8) and ethyl butanoate (C6) in SPI and water showed that an increase of ester chain length brought about greater binding affinity with the soy protein, which agreed with the results of Semenova, Antipova, Misharina, and Golovnya (2002).

In xanthan gum solution, significant differences ( $p < 0.05$ ) were observed compared to water for all flavor compounds except limonene. The apparent decrease of flavor compounds release induced by hydrocolloids is usually attributed to diffusion phenomena (Einhorn-Stoll & Drusch, 2015) or molecular interactions (Bylaite et al., 2005). In our experiments, the equilibrium of the system was reached; there should be no more diffusion phenomena and the molecular binding interactions (hydrogen bonding, Van der Waals forces, hydrophobic interactions or molecular inclusion) between flavor compounds and hydrocolloids might be the predominant determinant. Nevertheless, (Z)-3-hexenyl acetate in xanthan gum solution was obviously released ( $p < 0.05$ ). This effect could be attributed to a salting-out phenomenon revealing competition between (Z)-3-hexenyl acetate and the macromolecules of xanthan gum to bind water molecules. The same behavior was also reported for 2-butanone and 1-hexanol in maltodextrin solutions (Jouquand, Ducruet, & Giampaoli, 2004), and for ethyl butanoate and hexenal in custard (Martuscelli et al., 2008).

Compared to SPI solution, the xanthan gum solution showed significantly higher partition coefficients of flavor compounds except (Z)-3-hexen-1-ol (Table 2). Once the presence of xanthan gum in the SPI solution brought about a significant reduction ( $p < 0.05$ ) of partition coefficients for ethyl hexanoate, (Z)-3-hexen-1-ol and limonene, while for diacetyl, a significant enhancement ( $p < 0.05$ ) of partition coefficient was observed. This could be attributed to hydrophobic interaction or hydrogen bonding between flavor compounds and matrices. Also, the affinity of SPI with flavor compounds might be altered by a conformational change as xanthan gum adsorbs onto the protein, which results in the exposure of the hydrophobic binding sites in protein (Mao, Boiteux, Roos, & Miao, 2014).

### 3.3. Effect of xanthan gum concentration

The influence of xanthan gum on the release of flavor compounds was assessed by measuring the partitioning of flavor compounds in the SPI solutions containing 0%, 0.05% and 0.1% (w/w) xanthan gum. From Fig. 1, the extent of retention of flavor compounds depended on the concentration of xanthan gum and the physicochemical properties of flavor compounds.

**Table 2**  
Gas-matrix partition coefficients ( $K \times 100$ ) of flavor compounds in water, 2% (w/w) SPI, 0.1% (w/w) xanthan gum solutions and their mixtures calculated by PRV method at 37 °C and pH 7.0.

	Water	SPI	Xanthan gum	SPI + xanthan gum
Ethyl hexanoate	24.59 ± 1.23 <sup>a</sup>	10.62 ± 0.45 <sup>b</sup>	20.33 ± 0.90 <sup>c</sup>	5.52 ± 0.57 <sup>d</sup>
(Z)-3-hexenyl acetate	14.60 ± 0.96 <sup>a</sup>	10.61 ± 0.07 <sup>b</sup>	20.38 ± 0.00 <sup>c</sup>	10.23 ± 0.27 <sup>b</sup>
Ethyl 2-methylbutanoate	51.96 ± 1.22 <sup>a</sup>	17.40 ± 0.68 <sup>b</sup>	28.78 ± 0.05 <sup>c</sup>	16.50 ± 0.07 <sup>b</sup>
Ethyl butanoate	9.95 ± 1.04 <sup>a</sup>	8.16 ± 0.36 <sup>b</sup>	8.86 ± 0.30 <sup>ab</sup>	8.02 ± 0.07 <sup>b</sup>
(Z)-3-hexen-1-ol	1.70 ± 0.10 <sup>a</sup>	1.44 ± 0.02 <sup>b</sup>	1.21 ± 0.00 <sup>c</sup>	0.31 ± 0.02 <sup>d</sup>
Limonene	29.53 ± 1.56 <sup>a</sup>	24.79 ± 0.66 <sup>b</sup>	30.04 ± 0.44 <sup>a</sup>	9.92 ± 0.49 <sup>c</sup>
Diacetyl	0.27 ± 0.00 <sup>a</sup>	0.03 ± 0.00 <sup>b</sup>	0.08 ± 0.01 <sup>c</sup>	0.19 ± 0.01 <sup>d</sup>

The results are shown as a mean with standard deviation for triplicate samples. Values for different matrices with the same flavor compound have different small superscript letters (a, b, c and d) differ significantly ( $p < 0.05$ ).

**Table 3**

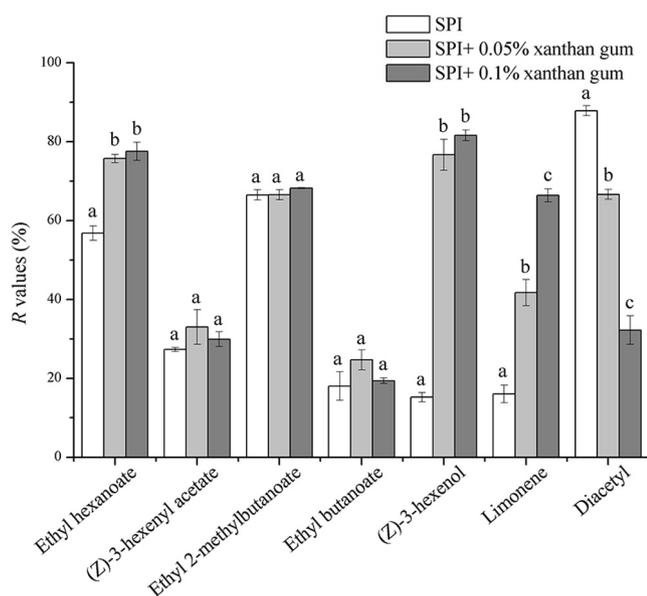
The ratio between partition coefficients of ester compound in different type of flavor compounds mixtures and single ester flavor compound. (A) water; (B) SPI solution; (C) mixture of SPI and xanthan gum solution.

Flavor compounds mixtures	Matrices	Ratio			
		Ethyl hexanoate	(Z)-3-hexenyl acetate	Ethyl 2-methylbutanoate	Ethyl butanoate
Ester + other three esters	A	0.21	0.08	0.03	0.39
	B	0.38	0.25	0.34	0.46
	C	3.13	1.70	2.61	1.57
Ester + non-ester volatiles <sup>a</sup>	A	0.93	1.09	0.81	1.55
	B	0.38	1.56	0.59	2.10
	C	3.32	2.57	2.91	1.60
Ester + less-volatiles <sup>b</sup>	A	1.34	4.81	0.38	3.09
	B	1.28	3.92	1.93	1.87
	C	4.62	2.45	1.14	3.35
Ester + other compounds <sup>c</sup>	A	1.36	2.10	0.71	1.57
	B	0.96	5.03	0.63	3.18
	C	6.13	5.27	1.26	2.92
Strawberry flavoring	A	0.44	0.78	0.51	0.56
	B	0.84	1.86	0.62	1.61
	C	5.54	2.12	1.27	2.24

<sup>a</sup> Mixture of non-ester volatiles including (Z)-3-hexen-1-ol, limonene and diacetyl.

<sup>b</sup> Mixture of less-volatiles including  $\gamma$ -decalactone, methyl cinnamate, hexanoic acid, 2-methylbutyric acid and furaneol.

<sup>c</sup> The mixture of other compounds including non-ester volatiles and less-volatiles mentioned above.



**Fig. 1.** Retention (%) of flavor compounds at 37 °C and pH 7.0 in 2% (w/w) SPI solutions containing 0%, 0.05% and 0.1% (w/w) xanthan gum. Different letters indicate significant difference for each flavor compound ( $p < 0.05$ ).

Xanthan gum is an anionic polysaccharide which has a main chain consisting of a linear backbone of 1, 4-linked  $\beta$ -D-glucose with a charged trisaccharide side chain on each second glucose residue (Jansson, Kenne, & Lindberg, 1975). It is a non-adsorbing polysaccharide with high viscosity and strong shear-thinning character (Qiu, Zhao, & McClements, 2015). The concentration of the xanthan has a minimal impact on the retention of esters, with the exception of ethyl hexanoate (57.76%). This phenomenon demonstrated that the behavior of esters, related to the nature of SPI, was not affected by the information of SPI and xanthan complexes, which is attributed to the electrostatic attraction of anionic groups on the xanthan molecules to cationic patches on the protein surface, or maybe due to hydrophobic or hydrogen interactions (Qiu et al., 2015). Esters can be advantageous for establishing Van der Waals interactions with proteins. In addition, they possess a large negatively charged surface area due to the two oxygen

atoms, which promote Keesom and Debye interactions (Ayed et al., 2014). As a result, only a few cases of significant differences in the retention behavior exist according to the matrix.

The retention of (Z)-3-hexen-1-ol (15.77%) significantly increased ( $p < 0.05$ ) with increasing xanthan gum concentrations and then kept unchanged at the highest xanthan gum concentration used. Alcohols are generally considered as best candidates to interact with polysaccharide by the way of hydrogen bonds (Semenova et al., 2002). Notably, with increasing xanthan gum concentration, the retention of limonene was significantly ( $p < 0.05$ ) enhanced, while diacetyl, a highly hydrophilic compound, was retained to a bare minimum. There was no doubt that hydrophobicity was an obvious parameter to explain the behavior of the flavor compounds in the presence of xanthan gum and SPI. This result was in accordance with the results of Arancibia, Castro, Jublot, Costell, and Bayarri (2015).

### 3.4. Effect of other flavor compounds on the interaction between ester compound and matrices

As food flavor is a complex mixture of many compounds in specific proportions, this can result in an alteration in perceived flavor if the proportion is changed (Schober & Peterson, 2004). Previous studies have found that esters are major compounds in strawberry flavor (Du, Plotto, Baldwin, & Rouseff, 2011; Nuzzi, Lo Scalzo, Testoni, & Rizzolo, 2008). Thus, it is worth understanding the behavior of esters (ethyl hexanoate, (Z)-3-hexenyl acetate, ethyl 2-methylbutanoate and ethyl butanoate) in different matrices and the effect of other strawberry flavor compounds. To gain the understanding of the effect of other flavor compounds on the interaction between specific ester and matrices, the mixtures of esters, volatiles and less-volatiles were studied in detail.

The presence of three other esters strongly influenced the interaction between ester compound and matrices. From the data related to the water system (Table 3), intense interactions within esters occurred and the release of ester compound was highly restrained, while less-volatiles and other compounds (the mixture of non-ester volatiles and less-volatiles) stimulated ester release, except ethyl 2-methylbutanoate. Also, the presence of non-ester volatiles was found to have slight impact on the behavior of ester compound. The release of ester compound was restrained in the strawberry flavoring, showing that the presence of other three

**Table 4**  
The ratio between partition coefficients of volatiles in different types of flavor compound mixtures and single flavor compound; (A) water; (B) SPI solution; (C) mixture of SPI and xanthan gum solution.

Flavor compound mixtures	Matrices	Ratio		
		(Z)-3-hexen-1-ol	Limonene	Diacetyl
Ethyl hexanoate + non-ester volatiles <sup>a</sup>	A	0.22	0.71	0.35
	B	0.26	0.73	0.24
	C	0.21	1.72	0.58
(Z)-3-hexenyl acetate + non-ester volatiles <sup>a</sup>	A	0.23	0.43	0.54
	B	0.25	0.48	0.59
	C	0.36	0.63	0.29
Ethyl 2-methylbutanoate + non-ester volatiles <sup>a</sup>	A	0.15	2.46	0.52
	B	0.24	0.29	0.67
	C	0.14	0.93	0.33
Ethyl butanoate + non-ester volatiles <sup>a</sup>	A	0.23	2.42	0.43
	B	0.20	0.60	0.34
	C	0.21	0.87	0.29
Ethyl hexanoate + other compounds <sup>b</sup>	A	0.20	2.21	0.38
	B	0.37	1.26	0.95
	C	0.25	0.61	0.65
(Z)-3-hexenyl acetate + other compounds <sup>b</sup>	A	0.17	0.46	0.35
	B	0.16	1.14	0.38
	C	0.15	1.00	0.25
Ethyl 2-methylbutanoate + other compounds <sup>b</sup>	A	0.26	0.90	0.51
	B	0.12	0.44	0.54
	C	0.14	0.95	0.13
Ethyl butanoate + other compounds <sup>b</sup>	A	0.20	0.71	0.39
	B	0.19	0.48	0.29
	C	0.19	1.80	0.57
Strawberry flavoring	A	0.14	0.96	0.31
	B	0.13	1.35	0.28
	C	0.11	0.52	0.84

<sup>a</sup> The mixture of non-ester volatiles including (Z)-3-hexen-1-ol, limonene and diacetyl.

<sup>b</sup> The mixture of other compounds including other volatiles mentioned above and  $\gamma$ -decalactone, methyl cinnamate, hexanoic acid, 2-methylbutyric acid and furaneol.

esters played a critical role in the behavior of the ester compound. Similar results to the water system were observed in the SPI system, in that there were strong interactions within esters and the release of ester compound was reduced. However, the inhibiting effect was lower than in the water systems, indicating that SPI could weaken the interaction within ester compounds to some extent. Although the interactions with non-ester volatiles, less-volatiles and their mixture were complicated and no universal laws were discovered for the behavior of ester compound in SPI system, the ratio of ester compounds in SPI was higher than in water in general. As for the mixture of SPI and xanthan gum system, the presence of three other esters had significant impact on the interaction between ester compound and the matrix. More specifically, the release of all esters was promoted more or less by other flavor compounds in the mixture of SPI and xanthan gum, indicating that the addition of xanthan gum in soy beverage on one hand may lead to flavor imbalance, on the other hand to improve the intensity of strawberry flavor. The phenomenon could be illustrated by the existence of a limited number of free space or absorption sites within the polymer (Johansson & Leufven, 1997).

As for the behavior of other volatiles ((Z)-3-hexen-1-ol, limonene and diacetyl) in the presence of esters and less-volatiles, the release of (Z)-3-hexen-1-ol and diacetyl was restrained in water, SPI as well as the mixture of SPI and xanthan gum solutions (Table 4). The above results demonstrated that presence of SPI or and xanthan gum could bring about an imbalance of the strawberry flavor.

#### 4. Conclusions

Both SPI and xanthan gum affected the release of flavor compounds. The release of volatiles was restrained in SPI, xanthan gum and the mixture of SPI and xanthan gum solutions compared with water alone. Increasing xanthan gum concentrations could

change the retention of (Z)-3-hexen-1-ol, limonene and diacetyl significantly, with less impact on the retention of four ester compounds. The presence of other flavor compounds strongly influenced the release of the individual ester in different matrices. The large magnitude of the variation of ratio of the esters occurred in SPI solution and the mixture with xanthan gum, compared with the behavior of esters in water, demonstrating that the addition of SPI and xanthan gum could bring about the imbalance of the strawberry flavor profile. This finding provides an important understanding of how ester release can be controlled using esters in a food system. To maximize the strawberry flavor perception in formulated soy protein beverage, the interactions of flavor compounds and the effects of inhibition or promotion on the release of flavor compounds should be further taken into account.

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#### References

- Arancibia, C., Castro, C., Jublot, L., Costell, E., & Bayarri, S. (2015). Colour, rheology, flavour release and sensory perception of dairy desserts. Influence of thickener and fat content. *LWT – Food Science and Technology*, 62(1), 408–416.
- Arora, A., & Damodaran, S. (2010). Competitive binding of off-Flavor compounds with soy protein and  $\beta$ -cyclodextrin in a ternary system: A model study. *Journal of the American Oil Chemists' Society*, 87(6), 673–679.
- Ayed, C., Lubbers, S., Andriot, I., Merabtine, Y., Guichard, E., & Tromelin, A. (2014). Impact of structural features of odorant molecules on their retention/release behaviours in dairy and pectin gels. *Food Research International*, 62, 846–859.
- Bylaite, E., Adler-Nissen, J., & Meyer, A. S. (2005). Effect of xanthan on flavor release from thickened viscous food model systems. *Journal of Agricultural and Food Chemistry*, 53(9), 3577–3583.

- Boland, A., Delahunty, C., & Vanruth, S. (2006). Influence of the texture of gelatin gels and pectin gels on strawberry flavour release and perception. *Food Chemistry*, 96(3), 452–460.
- Damodaran, S., & Kinsella, J. E. (1980). Flavor protein interactions. Binding of carbonyls to bovine serum albumin: Thermodynamic and conformational effects. *Journal of Agricultural and Food Chemistry*, 28(3), 567–571.
- Decourcelle, N., Lubbers, S., Vallet, N., Rondeau, P., & Guichard, E. (2004). Effect of thickeners and sweeteners on the release of blended aroma compounds in fat-free stirred yoghurt during shear conditions. *International Dairy Journal*, 14(9), 783–789.
- Du, X., Plotto, A., Baldwin, E., & Rouseff, R. (2011). Evaluation of volatiles from two subtropical strawberry cultivars using GC-olfactometry, GC-MS odor activity values, and sensory analysis. *Journal of Agricultural and Food Chemistry*, 59(23), 12569–12577.
- Einhorn-Stoll, U., & Drusch, S. (2015). Methods for investigation of diffusion processes and biopolymer physics in food gels. *Current Opinion in Food Science*, 3, 118–124.
- Endo, H., Ohno, M., Tanji, K., Shimada, S., & Kaneko, K. (2005). Effect of heat treatment on the lipid peroxide content and Aokusami (beany flavor) of soymilk. *Food Science and Technology Research*, 10(3), 328–333.
- Evageliou, V., & Patsiakou, A. (2014). The effect of sugars on the retention of ethyl butyrate by gellan gels. *Food Chemistry*, 157, 252–256.
- Evageliou, V., Papastamopoulou, K., Frantzeskaki, D., & Christodoulidou, C. C. (2015). Retention of esters by gellan and pectin solutions or their mixtures. *Food Hydrocolloids*, 51, 54–59.
- Feng, J., & Xiong, Y. L. (2003). Interaction and functionality of mixed myofibrillar and enzyme-hydrolyzed soy proteins. *Journal of Food Science*, 68(3), 803–809.
- González-Tomás, L., Bayarri, S., Taylor, A. J., & Costell, E. (2007). Flavour release and perception from model dairy custards. *Food Research International*, 40(4), 520–528.
- Heilig, A., Cetin, S., Erpenbach, K., Hohn, J., & Hinrichs, J. (2011). Inherent and process-induced hydrophobicity influences aroma retention in fat-free dairy matrices. *International Dairy Journal*, 21(9), 696–702.
- Jansson, P.-E., Kenne, L., & Lindberg, B. (1975). Structure of the extracellular polysaccharide from *Xanthomonas campestris*. *Carbohydrate Research*, 45(1), 275–282.
- Johansson, F., & Leufven, A. (1997). Concentration and interactive effects on the sorption of aroma liquids and vapors into polypropylene. *Journal of Food Science*, 62(2), 355–358.
- Jouquand, C., Ducruet, V., & Giampaoli, P. (2004). Partition coefficients of aroma compounds in polysaccharide solutions by the phase ratio variation method. *Food Chemistry*, 85(3), 467–474.
- Kühn, J., Zhu, X. Q., Considine, T., & Singh, H. (2007). Binding of 2-nonanone and milk proteins in aqueous model systems. *Journal of Agricultural and Food Chemistry*, 55(9), 3599–3604.
- Kühn, J., Delahunty, C. M., Considine, T., & Singh, H. (2009). In-mouth flavour release from milk proteins. *International Dairy Journal*, 19(5), 307–313.
- Lafarge, C., Cayot, N., Holy, C., Goncalves, L., Chassefont, C., & Le Bail, P. (2014). Effect of konjac glucomannan addition on aroma release in gels containing potato starch. *Food Research International*, 64, 412–419.
- Lubbers, S., Decourcelle, N., Martinez, D., Guichard, E., & Tromelin, A. (2007). Effect of thickeners on aroma compound behavior in a model dairy gel. *Journal of Agricultural and Food Chemistry*, 55(12), 4835–4841.
- Mao, L., Boiteux, L., Roos, Y. H., & Miao, S. (2014). Evaluation of volatile characteristics in whey protein isolate-pectin mixed layer emulsions under different environmental conditions. *Food Hydrocolloids*, 41, 79–85.
- Martuscelli, M., Savary, G., Pittia, P., & Cayot, N. (2008). Vapour partition of aroma compounds in strawberry flavoured custard cream and effect of fat content. *Food Chemistry*, 108(4), 1200–1207.
- Moon, S.-Y., & Li-Chan, E. C. Y. (2007). Assessment of added ingredient effect on interaction of simulated beef flavour and soy protein isolate by gas chromatography, spectroscopy and descriptive sensory analysis. *Food Research International*, 40(10), 1227–1238.
- Nuzzi, M., Lo Scalzo, R., Testoni, A., & Rizzolo, A. (2008). Evaluation of fruit aroma quality: comparison between gas chromatography-olfactometry (GC-O) and odour activity value (OAV) aroma patterns of strawberries. *Food Analytical Methods*, 1(4), 270–282.
- Philippe, E., Seuvre, A. M., Colas, B., Langendorff, V., Schippa, C., & Voilley, A. (2003). Behavior of flavor compounds in model food systems: A thermodynamic study. *Journal of Agricultural and Food Chemistry*, 51(5), 1393–1398.
- Qiu, C., Zhao, M., & McClements, D. J. (2015). Improving the stability of wheat protein-stabilized emulsions: Effect of pectin and xanthan gum addition. *Food Hydrocolloids*, 43, 377–387.
- Savary, G., Hucher, N., Petibon, O., & Grisel, M. (2014). Study of interactions between aroma compounds and acacia gum using headspace measurements. *Food Hydrocolloids*, 37, 1–6.
- Schober, A. L., & Peterson, D. G. (2004). Flavor release and perception in hard candy: Influence of flavor compound-compound interactions. *Journal of Agricultural and Food Chemistry*, 52(9), 2623–2627.
- Semenova, M. G., Antipova, A. S., Misharina, T. A., & Golovnya, R. V. (2002). Binding of aroma compounds with legumin. I. Binding of hexyl acetate with 11s globulin depending on the protein molecular state in aqueous medium. *Food Hydrocolloids*, 16(6), 557–564.
- Tromelin, A., Ayed, C., Lubbers, S., Pages-Helary, S., Andriot, I., & Guichard, E. (2012). Proposed alternative phase ratio variation method for the calculation of liquid-vapour partition coefficients of volatiles. *Journal of Chromatography A*, 1263, 158–168.
- Van Durme, J., & Werbrouck, B. (2015). Phase ratio variation approach for the study of partitioning behavior of volatile organic compounds in polymer sample bags: Nalophan case study. *Environmental Science and Pollution Research*, 22(14), 11067–11075.
- Vidrih, R., Zlatic, E., & Hribar, J. (2009). Release of strawberry aroma compounds by different starch-aroma systems. *Czech Journal of Food Sciences*, 27, S58–S61.
- Wang, K., & Arntfield, S. D. (2014). Binding of carbonyl flavours to canola, pea and wheat proteins using GC/MS approach. *Food Chemistry*, 157, 364–372.
- Wang, K., & Arntfield, S. D. (2015). Binding of selected volatile flavour mixture to salt-extracted canola and pea proteins and effect of heat treatment on flavour binding. *Food Hydrocolloids*, 43, 410–417.
- Zafeiropoulou, T., Evageliou, V., Gardeli, C., Yanniotis, S., & Komaitis, M. (2012). Retention of selected aroma compounds by gelatine matrices. *Food Hydrocolloids*, 28(1), 105–109.