



## Analytical Methods

# Response surface methodology and multivariate analysis of equilibrium headspace concentration of orange beverage emulsion as function of emulsion composition and structure

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## ABSTRACT

The influence of emulsion composition (i.e. Arabic gum, xanthan gum and, orange oil) and structural emulsion properties (i.e. average droplet size and apparent viscosity) on equilibrium headspace concentration of beverage emulsions was investigated. Increase in average droplet size led to increase the equilibrium headspace concentration of more hydrophilic volatile compounds (i.e. lower log  $P$ ) such as ethyl acetate and octanal, but decrease in more hydrophobic volatile compounds such as 3-carene, myrcene and limonene. In most cases, apparent viscosity had significant positive effect on equilibrium headspace concentration. Principle component analysis (PCA) score discriminated the beverage emulsions containing the same orange oil content but different contents of emulsifiers in different classes, thus indicating the significant ( $p < 0.05$ ) effect of emulsifier fraction on equilibrium headspace concentration. Beverage emulsion containing 22.2% (w/w) Arabic gum, 0.52% (w/w) xanthan gum and 14.21% (w/w) orange oil was estimated to provide the highest equilibrium headspace concentration.

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## 1. Introduction

Flavours are one of the most important components responsible for the overall distinctive sensory properties and organoleptic attributes of food stuff. For flavouring of emulsion and dispersion systems, major difficulties are associated with the reversibility of binding of flavour compounds by various components, including the bulk phases, interfacial layers, macromolecules, macromolecular aggregates, inter-biopolymer and other complexes. Physico-chemical interactions between flavour compounds and food components can affect flavour compound migration in foods by modifying the nature and number of free binding sites as well as the affinity of the flavour compounds (Landy, Druaux, & Voilley, 1995; Nongonierma, Colas, Springett, Le Quééré, & Voilley, 2007).

Flavour release is defined as a flavour compound transport process from the matrix to the vapour phase (Taylor, 2002). Both binding and release of flavours are influenced by the composition and structure of foods during processing and storage (Bakker, 1995). Flavor release from emulsions is mainly dependent on the affinity of volatile compounds for the liquid phases but could also be affected by the structure of the emulsions. The emulsion structure is characterised by the nature of the dispersed phase (water or oil),

the surface area of the lipid–water interface, and the nature and amount of the surface-active agent adsorbed at the interface (Charles, Rosselin, Beck, Sauvageot, & Guichard, 2000). The release of volatile compounds from the emulsion system can occur through volatilisation or reaction with other components, thus the changes in emulsion matrix and structure could modify odorant interaction and release.

Beverage emulsions are a unique class of the flavoured-colloid systems that are prepared in a concentrated form and then diluted several hundred times in sugar/acid solution in order to provide an opaque appearance and suitable aroma in either carbonated or non-carbonated beverage. The addition of beverage emulsion to the soft drink changes the sensory properties and organoleptic attributes of the beverage phase, thus altering volatile compound partition. Thus, the release of volatile flavour compounds from beverage emulsion during processing and storage plays an important role for the manufacturer in deciding the degree of satisfaction and the overall acceptability of the flavoured-beverage emulsion in the finished emulsion-based product (i.e. soft drink).

Food hydrocolloids are high-molecular weight polysaccharides widely used for the control of microstructure, texture and shelf life in colloid systems. The addition of hydrocolloid to food products may influence the rate and intensity of flavour release through a physical entrapment of flavour molecules within the emulsion matrix, or through a specific or non-specific binding of flavour molecules (Carr et al., 1996; Secouard, Malhiac, Grisel, & Decroix, 2003).

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They can also affect the volatile flavour release through either physical entrapment of flavour molecules within emulsion matrix, or by modifying the emulsion structure (i.e. droplet size and viscosity).

It should be noted that the effect of main emulsion composition on the other physicochemical properties of beverage emulsions such as zeta potential, conductivity, mobility, polydispersity index, physical stability, cloudiness, turbidity loss rate, pH and density were investigated in our previous studies (Mirhosseini, Tan, Hamid, & Yusof, 2007a; Mirhosseini, Tan, Hamid, & Yusof, 2008a; Mirhosseini, Tan, Hamid, & Yusof, 2008b). As shown in our previous study, the proportion of main emulsion components especially the content of main emulsifier fraction (i.e. Arabic gum) significantly ( $p < 0.05$ ) affected the emulsion stability and the rheological properties of beverage emulsion. On the other hand, the physical properties of beverage emulsion such as cloudiness and turbidity loss rate were significantly ( $p < 0.05$ ) influenced by the oil phase content.

In this study, a three-factor central composite design (CCD) was used to investigate the effect of emulsifier fraction content (i.e. Arabic gum (10.78–22.22% w/w) and xanthan gum (0.24–0.56% w/w)), dispersed phase content (i.e. orange oil (8.73–15.27% w/w)), average droplet size and apparent viscosity on the equilibrium headspace concentration of orange beverage emulsion. Headspace solid phase microextraction (HS-SPME) was employed for quantitative analysis of the equilibrium headspace concentration of beverage emulsion. HS-SPME has also been used as a fast alternative technique to determine the flavour release in previous studies (Nguyen, Campi, Jackson, & Patti, 2009; Yu, Sun, Tian, & Qu, 2008). In this study, the main objectives of present study were to (1) investigate the effect of main emulsion composition and matrix structure on the equilibrium headspace concentration, (2) determine the release pattern and equilibrium headspace concentration of target flavour compounds in the headspace of orange beverage emulsion and (3) determine the optimum concentration level of main emulsion components leading to the minimum and maximum volatile flavour release. The present study shows us how the interaction between emulsifier fraction and orange oil compounds affects the volatile flavour release from the concentrate beverage emulsion. These optimisation studies allow the manufacturers to develop the pre-formulation of the beverage emulsion with desirable volatile flavour release and physicochemical properties.

## 2. Materials and methods

### 2.1. Materials

Ethyl acetate (99%),  $\alpha$ -pinene (99.5%), ethyl butyrate (99.7%),  $\beta$ -pinene (98.5%), 3-carene (98.5%), myrcene (95%), limonene (99%),  $\gamma$ -terpinene (98.5%), octanal (98%), decanal (95%), linalool (95%), octanol (95%) and citral (95%) (neral and geranial) were supplied by Fluka (Buch, Switzerland). Arabic gum (food grade) was provided by Colloides Naturels International Co. (Rouen, France). Xanthan gum was donated by CP Kelco (San Diego, CA, USA). Citric acid, sodium benzoate and potassium sorbate (p.a.  $\geq 95\%$ ) were purchased from Fisher Scientific (Pittsburgh, PA, USA). Cold pressed orange oil was provided by Danisco (Aarhus, Denmark).

### 2.2. Sample preparation

In the preset study, 20 orange beverage emulsions containing Arabic gum (10.78–22.22% w/w), xanthan gum (0.24–0.56% w/w), orange oil (8.73–15.27% w/w), sodium benzoate (0.1% w/w), potassium sorbate (0.1% w/w), citric acid (0.5% w/w) and deionized

water were prepared based on three-factor central composite design (CCD). To prepare the water phase, sodium benzoate, potassium sorbate and citric acid were sequentially dispersed in deionized water (60 °C) using a high speed blender (Waring blender 32BL80, New Hartford, CO, USA). While mixing the mixture, Arabic gum was gradually added to the deionized water (60 °C) and mixed for 3 min to facilitate hydration. The gum solution was kept overnight at room temperature to fully hydrate (Buffo, Reineccius, & Oehlert, 2001).

Xanthan gum solution was prepared separately by dissolving xanthan gum in deionized water and then mixed with hydrated Arabic gum solution by using a high speed blender. While mixing the water phase, the cold pressed orange oil was gradually dispersed in water phase to provide an initial coarse emulsion. Fine emulsification (i.e. small emulsion droplet size of  $< 1 \mu\text{m}$  and narrow particle size distribution) was achieved by subjecting pre-emulsions to high shear homogenizer (Silverson L4R, Buckinghamshire, UK) for 1 min and then passed through a high pressure homogenizer (APV, Crawley, UK) for three passes (30, 28 and 25 MPa).

### 2.3. Apparent viscosity

The apparent viscosity of beverage emulsions was measured immediately after the sample preparation by means of a steady stress Brookfield viscometer (Brookfield DV-II+, Middleboro, MA, USA) equipped with the LV spindles. The principal of viscometer operation is to immerse a spindle in the test fluid and then drive it through a calibrated spring. The viscous drag of the fluid against the spindle is measured by the spring deflection. The apparent viscosity of fixed volume of emulsion in a 600 ml beaker was measured in duplicate at 60 rpm and then the average of two individual measurements was taken for data analysis. The spindle depth was kept constant throughout the viscosity measurements. The procedure for the measurement of apparent viscosity has been described by previous study (Ibanoğlu, 2002). Apparent viscosity measurement range of a Brookfield programmable viscometer DV-II+ appeared in unit of  $\text{MPa} \times \text{s}$ .

### 2.4. Average droplet size and polydispersity index (PDI)

Average droplet size and PDI of orange beverage emulsions were evaluated immediately after sample preparation by using a Malvern zeta and particle size analyser (Malvern series ZEN 3500, Malvern Instruments Ltd., Worcester, UK). To avoid multiple scattering effects, the beverage emulsions were diluted (1:100; approximately 250 ml) with deionized water prior to analysis; then the diluted emulsions were directly placed into the module. A laser beam was directed through the diluted samples, scattered by the droplets in a characteristic pattern dependent on their size and subsequently detected by an array of photodiodes located behind the cuvette. The measurement range of a Malvern zeta sizer appeared in the units of nm for average droplet size. The PDI was calculated as the best fit between the measured scattered pattern and the one predicted by the light scattering theory. The measurements were reported as average of three individual injections, with four readings made per injection.

### 2.5. HS-SPME procedure

For HS-SPME analysis, 10 g of each orange beverage emulsion was transferred into a 20 ml serum vial containing a micro stirring bar. Subsequently, the vial was sealed with a Teflon-lined septa and screw cap that was immersed in a water bath at 45 °C. The SPME fibre coated with CAR/PDMS was manually exposed to the sample headspace for 30 min at 45 °C under agitating mode to

rapidly reach equilibrium condition. Finally, the fibre was immediately introduced into the GC injection port and held for 8 min to be completely desorbed the volatile compounds. The measurements were reported as the average of two individual injections.

## 2.6. Instruments

In the present study, the volatile flavour compounds were initially detected by using a Hewlett–Packard 6890N GC system (Wilmington, DE) equipped with Electron Ionization–Time-of-Flight Mass Spectrometer (TOFMS, Pegasus III, Leco Corp., MI, USA) and a DB-Wax column (J&W Science, i.d. = 0.25 mm, length = 30 m, film thickness = 0.25  $\mu$ m, Supelco, MA, USA). For qualitative analysis, the injections were performed at split mode (1:200) with the injector temperature held at 250 °C for 5 min. The GC injection port was equipped with a 0.75 mm i.d. liner to minimise peak broadening. Oven temperature was programmed at 45 °C for 5 min, then ramped to 51 °C at 1 °C/min and held for 5 min at 51 °C then increased to 160 °C at 5 °C/min. Oven temperature was finally raised to 250 °C at 12 °C/min and held for 15 min at final temperature (Mirhosseini, Yusof, Hamid, & Tan, 2007b). Detector and injector temperatures were set at 270 °C.

After qualitative analysis, the hydrophobicity of the target volatile compounds was estimated by determining the log *P* according to the method described by Rekker (1977). The hydrophobicity strength of the inner medium is defined as relative to *n*-octanol water partition coefficient. Amongst the thermodynamic parameters that affect flavour release, log *P* (indicator of hydrophobicity and therefore polarity) is key parameter describing odorant behaviour. Subsequently, the peaks were verified by running the known standard solutions and samples, respectively. For the equilibrium headspace analysis of target volatile flavour released from the beverage emulsions, Hewlett–Packard 6890N GC system (Wilmington, DE) equipped with a flame ionisation detector (FID) was employed. Helium was used as the carrier gas with flow rate of 1.4 ml/min and injections were performed at the splitless mode. Almost the same experimental conditions employed for the qualitative analysis using GC–TOFMS were applied for the quantitative equilibrium headspace analysis using GC–FID.

## 2.7. Experimental design and data analysis

The effect of main emulsion components namely Arabic gum (0.78–22.22% w/w,  $x_1$ ), xanthan gum (0.24–0.56% w/w,  $x_2$ ) and orange oil (8.73–15.27% w/w,  $x_3$ ) on the equilibrium volatile headspace concentration of orange beverage emulsion was evaluated by using the central composite design (CCD). Thus, 20 treatments were assigned based on the CCD with three independent variables at five levels of each variable involving eight factorial points, six star points and six centre points. The use of blocked design with orthogonal blocking allows the estimation of individual and interaction factor effects independently of block effects. Blocks are assumed to have no impact on nature and shape of response surface.

The peak area of each volatile flavour compound and total peak area were considered as response variables. The terms statistically found non-significant ( $p > 0.05$ ) were dropped from the initial models and the experimental data was refitted only to the significant ( $p < 0.05$ ) parameters in order to obtain the final reduced model. It should be noted that some variables were kept in the reduced model despite non-significance ( $p > 0.05$ ) as a quadratic or interaction variable effect containing this variable was significant ( $p < 0.05$ ). Minitab v. 13.2 statistical package (Minitab Inc., State College, PA, USA) was used for the creation of the experimental design and data analysis.

## 2.8. Multivariate analysis

Multivariate analysis was carried out using principal component analysis (PCA) based on the correlation matrix to (1) elaborate the mutual relationship amongst the equilibrium headspace concentration of the target volatile compounds and (2) obtain an overview of how the samples were correlated to each other with regard to volatile flavour concentration in the equilibrium headspace. In multivariate analysis, correlation matrix was applied by using Minitab software so that the data was autoscaled by variable to give same weight to all components.

## 2.9. Optimisation and validation procedures

Minitab software provides the graphical and numerical multiple optimisation procedures in order to identify the combination of input variable settings that jointly optimise a set of responses. For graphical optimisation procedure, the final reduced models were expressed as three dimensional (3D) response surface plots to better visualise the significant ( $p < 0.05$ ) interaction effect of main emulsion components on the equilibrium headspace concentration of volatile flavour compounds released from the orange beverage emulsions. Optimum levels of independent variables resulting in main response goals were pre-established by superimposing all response surface plots.

For numerical multiple optimisations, the response optimiser was applied by using the Minitab software for determining the exact optimum level of independent variables leading to the least and highest equilibrium headspace concentration. This numerical response optimisation allows us to interactively change the input variable settings in order to perform sensitivity analyses and possibly improve the initial solution. The experimental data was compared with the fitted values predicted by the response regression equations in order to verify the accuracy of final reduced models.

## 3. Results and discussion

### 3.1. Preliminary study

The volatile compounds including ethyl acetate,  $\alpha$ -pinene, ethyl butyrate,  $\beta$ -pinene, 3-carene, myrcene, limonene,  $\gamma$ -terpinene, octanal, decanal, linalool, neral and geranial were composed of more than 98% of total flavour compounds of cold pressed orange oil (data not shown). Twelve volatile flavour compounds were selected from various chemical classes such as ester, monoterpene hydrocarbon, alcohol and aldehyde groups representing different hydrophobicity value (log *P*, 0.68–4.65) (Table 1). As reported in previous study (Hognadottir & Rouseff, 2003), these volatile flavour compounds were chosen as the representative of main monoterpene hydrocarbons, aldehydes, alcohols, esters, terpeneols present in cold pressed orange oils. Hence, the peak area of target flavour compounds was considered as response variables in further optimisation study.

**Table 1**  
Levels of independent variables established according to the central composite design (CCD).

Variable	Independent variable levels				
	Low	Centre	High	Axial ( $-\alpha$ )	Axial ( $+\alpha$ )
Arabic gum content (% w/w)	13.00	16.50	20.00	10.78	22.22
Xanthan gum content (% w/w)	0.30	0.40	0.50	0.24	0.56
Orange oil content (% w/w)	10.00	12.00	14.00	8.73	15.27

### 3.2. Effect of emulsion structure on equilibrium headspace concentration

As shown in Table 1, the main linear effect of average droplet size showed the significant ( $p < 0.05$ ) negative effect on equilibrium headspace concentration of most of target volatile compounds released from the beverage emulsions (Table 1). The results indicated that effect of emulsion structure on equilibrium headspace concentration of target volatile compounds depended on the hydrophobicity of the volatile compounds. As a rule, the increase in average droplet size thereby decrease in oil–water the interfacial surface area may enhance the mass transfer rate of the hydrophilic compounds (with low  $\log P$ ) from oil phase to aqueous phase. It can reduce the mass transfer rate of the hydrophobic volatile compounds (with higher  $\log P$ ).

As shown in Table 1, the equilibrium headspace concentration of more hydrophilic volatile compounds (with lower  $\log P$ ) such as ethyl acetate and octanal was higher in the beverage emulsion with the larger droplet size. The increase in equilibrium headspace concentration of more hydrophilic compounds (with lower  $\log P$ ) might be explained by lower resistance to mass transfer in the aqueous phase of the beverage emulsion when the droplet size increased and vice versa. Conversely, the equilibrium headspace con-

centrations of more hydrophobic volatile compounds (with higher  $\log P$ ) such as 3-carene, myrcene and limonene increased when the droplet size decreased (Table 1). This observation has also been reported by previous researchers (Charles et al., 2000; Van Ruth, King, & Delahunty, 2000). Van Ruth et al. (2000) also demonstrated that the increase of particle diameter increased the release intensity of aroma compounds from the o/w emulsions containing Tween 20. The authors described that the changes in particle diameter had a considerable effect on the thermodynamic component of aroma release. Charles et al. (2000) also found that the volatile release content of citrus aromas from salad dressings significantly ( $p < 0.05$ ) increased as droplet size increased.

In most cases except for octanal and linalool, the equilibrium headspace concentration was positively influenced by the significant ( $p < 0.05$ ) effect of apparent viscosity (Table 1). Most of target volatile flavour compounds such as ethyl acetate,  $\alpha$ -pinene, 3-carene, myrcene, limonene,  $\gamma$ -terpinene, decanal, neral and geranial were better released from the orange beverage emulsion, which was the more viscous (Table 1). Thus, the increase in apparent viscosity led to increase of the product–air interface, thereby increasing the equilibrium headspace concentration of most of target volatile flavour compounds released from the beverage emulsion. On the other hand, the increase of viscosity is related to a change

**Table 2**  
Volatile flavour compounds of Valencia cold pressed orange oil identified by using HS-SPME–GC–MS.

No.	Compound	Similarity	Retention time (s)	Formula	Identification	FID area (%)	Log $P^e$
1	Ethenyl acetate	854	95.48	C <sub>4</sub> H <sub>6</sub> O <sub>2</sub>	RI	tr.	–
2	Ethyl acetate	823	98.34	C <sub>4</sub> H <sub>8</sub> O <sub>2</sub>	RI, MS	0.07	0.68
3	Ethyl propanoate	878	201.08	C <sub>5</sub> H <sub>10</sub> O <sub>2</sub>	RI	tr.	–
4	$\alpha$ -Pinene <sup>b,c,d</sup>	904	279.94	C <sub>10</sub> H <sub>16</sub>	RI, MS	0.63	3.94
5	Butanoic acid, methyl ester	894	299.98	C <sub>9</sub> H <sub>16</sub> O <sub>3</sub>	RI, MS	tr.	–
6	3-Methoxyhex-1 ene	896	303.38	C <sub>7</sub> H <sub>14</sub> O	RI	tr.	–
7	2-Undecen-4-ol	931	319.18	C <sub>11</sub> H <sub>22</sub> O	RI	tr.	–
8	Ethyl butyrate <sup>a,c</sup>	899	342.29	C <sub>6</sub> H <sub>12</sub> O <sub>2</sub>	RI, MS	0.86	1.82
9	$\beta$ -Pinene <sup>b,c,d</sup>	954	446.19	C <sub>10</sub> H <sub>16</sub>	RI, MS	0.14	4.30
10	3-Carene <sup>d</sup>	956	500.02	C <sub>10</sub> H <sub>16</sub>	RI, MS	0.10	4.38
11	Myrcene <sup>a,b,c,d</sup>	875	769.43	C <sub>10</sub> H <sub>16</sub>	RI, MS	1.30	4.58
12	(S)-Cinene or carvene,	945	799.34	C <sub>10</sub> H <sub>16</sub>	RI	tr.	–
13	Limonene <sup>a,b,c,d</sup>	967	823.76	C <sub>10</sub> H <sub>16</sub>	RI, MS	93.8	4.65
14	1,8-Cineole <sup>b</sup>	890	965.19	C <sub>10</sub> H <sub>18</sub> O	RI	tr.	–
15	Camphene	859	978.14	C <sub>10</sub> H <sub>16</sub>	RI	tr.	–
16	$\gamma$ -Terpinene <sup>b,c,d</sup>	887	990.57	C <sub>10</sub> H <sub>16</sub>	RI, MS	0.17	4.54
17	p-Cymene <sup>b,c,d</sup>	959	1318.92	C <sub>10</sub> H <sub>14</sub>	RI, MS	tr.	–
18	3,5-Dimethylanisole	893	1424.78	C <sub>9</sub> H <sub>12</sub> O	RI	tr.	–
19	Octanal <sup>a,b</sup>	829	1485.43	C <sub>8</sub> H <sub>16</sub> O	RI, MS	0.23	2.67
20	Limonene oxide, cis- <sup>d</sup>	967	1620.58	C <sub>10</sub> H <sub>16</sub> O	RI, MS	tr.	–
21	Linalool tetrahydride	997	1659.58	C <sub>10</sub> H <sub>22</sub> O	RI	tr.	–
22	Limonene oxide, trans- <sup>d</sup>	889	1680.98	C <sub>10</sub> H <sub>16</sub> O	RI	tr.	–
23	(R)-(+)-citronellal <sup>b,c,d</sup>	948	1706.13	C <sub>10</sub> H <sub>18</sub> O	RI, MS	tr.	–
24	Cyclododecanol	945	1724.18	C <sub>12</sub> H <sub>24</sub> O	RI	tr.	–
25	Decanal <sup>a,b,c,d</sup>	912	1754.12	C <sub>10</sub> H <sub>20</sub> O	RI, MS	0.12	3.88
26	2,4-Dimethyl-1-penten-3-ol	792	1911.93	C <sub>7</sub> H <sub>14</sub> O	RI	nd	–
27	(E)-Geranyl methyl ether <sup>c,d</sup>	824	1919.13	C <sub>11</sub> H <sub>20</sub> O	RI	nd	–
28	2-Methyl-1,5-heptadien-4-ol	719	1922.78	C <sub>8</sub> H <sub>14</sub> O	RI	nd	–
29	Hexanol	914	1927.87	C <sub>6</sub> H <sub>14</sub> O	RI	tr.	–
30	Linalool <sup>a,b,c,d</sup>	930	1930.20	C <sub>10</sub> H <sub>18</sub> O	RI, MS	0.96	3.15
31	$\alpha$ -Terpineol <sup>c</sup>	939	2148.73	C <sub>10</sub> H <sub>18</sub> O	RI, MS	tr.	–
32	1-Octanol <sup>b,d</sup>	943	2179.11	C <sub>8</sub> H <sub>18</sub> O	RI, MS	tr.	–
33	Neral <sup>b,c,d</sup>	897	2612.13	C <sub>10</sub> H <sub>16</sub> O	RI, MS	0.11	3.24
34	Geranial <sup>b,c,d</sup>	849	2656.74	C <sub>10</sub> H <sub>16</sub> O	RI, MS	0.16	3.26
35	Octanoic Acid	954	2699.77	C <sub>8</sub> H <sub>16</sub> O <sub>2</sub>	RI	tr.	–
36	Sorbic Acid	872	2708.43	C <sub>6</sub> H <sub>8</sub> O <sub>2</sub>	RI	tr.	–
37	n-Decanoic acid	923	2737.12	C <sub>10</sub> H <sub>20</sub> O <sub>2</sub>	RI	tr.	–
38	1-Butanol, 4-methoxy-	798	2787.13	C <sub>5</sub> H <sub>12</sub> O <sub>2</sub>	RI	tr.	–
39	Hexagol	784	2822.51	C <sub>12</sub> H <sub>26</sub> O <sub>7</sub>	RI	tr.	–

nd, not detected; tr., trace. RI, identification based on retention index. MS, identification based on comparison of mass spectra.

<sup>a</sup> Identified by Bovill (1996).

<sup>b</sup> Identified by Hognadottir and Rouseff (2003).

<sup>c</sup> Identified by Minh Tu et al. (2003).

<sup>d</sup> Identified by Shen, Mishra, Imison, Palmer & Fairclough (2002).

<sup>e</sup> Based on the method described by Rekker (1977).



in the solid-water ratio. The increase in apparent viscosity results in a lower amount of water phase for dissolution of the volatiles, thus increasing the relative concentration of volatile compounds in the water phase. This phenomenon leads to a higher tendency of volatile compounds in the vapour phase. As demonstrated in previous study (Otake, Roozen, & Burger 1998), the more viscous products had more volatile flavour release than less viscous products due to the increase of the product-air interface.

In most cases except for ethyl butyrate,  $\gamma$ -terpinene, decanal, nerol and geraniol, the increase in the equilibrium headspace concentration of target volatile flavour compounds was observed with increasing the PDI or size distribution range. Thus, the monodisperse emulsion structure with more homogeneity (i.e. low PDI) showed less equilibrium headspace concentration (or less volatile release intensity) compared to polydisperse emulsion matrix (i.e. high PDI) (Table 1). Amongst the interaction variable effects, the interaction effect of average droplet size and apparent viscosity showed the most significant ( $p < 0.05$ ) interaction effect on the equilibrium headspace concentration of most of target volatile flavour compounds; whilst the interaction effect of average droplet size and PDI had the least significant ( $p < 0.05$ ) interaction effect on the equilibrium headspace concentration (Table 1).

### 3.3. Response surface analysis

The response surface analysis indicated that the significant ( $p < 0.05$ ) second order polynomial response models with high coefficient of determination ( $R^2$ ) ranging from 0.805 to 0.952 were adequately fitted to the experimental data (Table 2). No significant ( $p > 0.05$ ) lack of fit was observed for the regression equations, thus ensuring a satisfactory fitness of response surface models to the experimental data (Table 2). Hence, at least 80% of the variation of equilibrium headspace concentration could be explained as a function of main emulsion components (Table 2). It should be noted that the reduced models may not be true beyond the ranges of the factors. As stated by Montgomery (2001), the reduced polynomial regression equations fitted to the experimental data was only a statistical empirical model in the selected ranges. Therefore, response models may not be extrapolated beyond these ranges.

As shown in Table 2, all main effects except for main linear effect of xanthan gum on  $\gamma$ -terpinene should be included in the final reduced models. They showed the most significant ( $p < 0.05$ ) effects on the equilibrium headspace concentration of target volatile flavour compounds. Amongst the target volatile compounds, the main linear effect of orange oil and its interaction effect with xanthan gum had the most significant ( $p < 0.05$ ) effects on the equilibrium headspace concentration of linalool. Conversely, the linear effect of Arabic gum showed the least significant ( $p < 0.05$ ) effect on the equilibrium headspace concentration of decanal (Table 2).

### 3.4. Effect of main emulsion components on equilibrium volatile headspace concentration

The significant ( $p < 0.05$ ) effect of the main emulsion components was observed on equilibrium headspace concentration of target volatile compounds released from the orange beverage emulsions (Table 2). In most cases except for myrcene and octanal, the equilibrium headspace concentration of target volatile flavour compounds significantly ( $p < 0.05$ ) decreased with increasing the Arabic gum content as main surface active agent in the beverage emulsion formulation (Table 2). This may be contributed to the presence of an arabinogalactan attached to a polypeptide backbone (AGP) in the molecular structure of Arabic gum. AGP complex has a coil conformation with a small radius of gyration and equivalent sphere hydrodynamic radius. The hydrophobic polypeptide chain is believed to bind the hydrophobic flavour compounds; whilst

hydrophilic arabinogalactan blocks are able to anchor the hydrophilic volatile flavour compounds. As volatile flavour compounds are known to interact with protein, thus the decreased equilibrium headspace concentrations induced by Arabic gum could be explained by the specific interaction of the target volatile compounds with AGP fragment of Arabic gum at the o/w interfacial area or in the aqueous phase. Adsorption of the protein segment at the interface could either mask the aroma binding sites or facilitate the access for aroma to the binding sites of the proteins. The protein segment adsorbed at the o/w interface can also act as a barrier and decrease the mass transfer rate of target volatile compounds through the oil-water interface, thereby possibly reducing equilibrium headspace concentration (Rogacheva, Espinoza-Diaz, & Voilley, 1999).

The main linear effect of xanthan gum had the significant ( $p < 0.05$ ) negative effect on the total flavour compounds and equilibrium headspace concentration of most of hydrophobic volatile flavour compounds (Table 2). The suppressive effect of xanthan gum on equilibrium headspace concentration of hydrophobic volatile flavour compounds such as limonene has been explained by different hypotheses. It may be interpreted by the reason that xanthan has more distinctive hydrophobic characteristic compared to other hydrocolloids, thus hydrogen bindings between xanthan gum and hydrophobic flavour compounds influence their equilibrium headspace concentration (Yven, Guichard, Giboreau, & Roberts, 1998). In fact, the bindings can occur in the hydrophobic zones. The formation of micelles with surfactants similarly can occur in xanthan gum solution through the intermolecular interactions between methyl groups of pyruvate function. This can favour a phase separation and the possible interactions occurred in the hydrophobic zones of xanthan gum/Arabic gum mixture. As also reported by Secouard, Grisel, and Malhiac (2007), the headspace concentration of limonene in the o/w emulsion depended on the xanthan gum concentration. The authors explained that limonene was mainly retained by the xanthan gum solution through steric phenomena. Bylaite, Adler-Nissen, and Meyer (2005) also observed that the release of limonene decreased due to interaction with xanthan matrix.

Xanthan gum has high molecular weight molecules (~3,000,000 Da) composed of a 1-4 linked  $\beta$ -D-glucose backbone substituted on every second unit with a charged trisaccharide side-chain composed of a residue of glucuronic acid between two mannose units. The terminal mannose unit may be substituted

**Table 3**  
Regression coefficients,  $R^2$ , probability values and lack of fit for the final reduced models.

Regression coefficient	Average droplet size (nm, $Y_3$ )	PDI ( $Y_4$ )	Viscosity ( $Y_2$ )
$b_0$	-71	-0.9730	458
$b_1$	54	0.0680	-47.4
$b_2$	-1726	1.2101	-567.8
$b_3$	122	0.0777	-13.3
$b_1^2$	-	-0.0009	2.5
$b_2^2$	-	-	1296.1
$b_3^2$	-3	-	-
$b_{12}$	38	-	-58.6
$b_{13}$	-4	-0.0032	-
$b_{23}$	151	-0.0812	91.2
$R^2$	0.996	0.851	0.981
Regression ( $P$ -value)	0.000 <sup>a</sup>	0.001 <sup>a</sup>	0.000 <sup>a</sup>
Lack of fit ( $F$ -value)	6.56	3.99	4.31
Lack of fit ( $p$ -value)	0.740 <sup>b</sup>	0.141 <sup>b</sup>	0.129 <sup>b</sup>

$b_1$ : The estimated regression coefficient for the main effects.  $b_{ij}$ : The estimated regression coefficient for the quadratic effects.  $b_{ij}$ : The estimated regression coefficient for the interaction effects. 1: Arabic gum; 2: xanthan gum; 3: orange oil.

<sup>a</sup> Significant ( $p < 0.05$ ).

<sup>b</sup> Non-significant ( $p > 0.05$ ).

by pyruvate acetate. An *O*-acetyl group is frequently present at the inner mannose (Secouard et al., 2007). Schorsh, Garnier, and Doublier (1997) explained that the pyruvate groups of xanthan gum play a major role in the exclusion phenomena between the hydrocolloid and volatile compounds. Consequently, this strongly affects the aroma release from the emulsion and dispersion systems. Xanthan gum structure has a large number of free carboxyl groups that cause great water absorption capacity. When xanthan gum is dispersed in water system, its complex molecules form complicated aggregates through hydrogen bonds and polymer entanglement. Because of its complicated network and entanglements, very little increase in xanthan gum content can reduce the overall equilibrium headspace concentration.

As shown in Tables 2 and 3, the independent variables exhibited the most significant ( $p < 0.05$ ) effect on the equilibrium headspace concentration of aldehyde compounds studied (i.e. octanal, decanal, neral and gernal), alcohol (i.e. linalool) and monoterpene hydrocarbons (i.e.  $\alpha$ -pinene,  $\beta$ -pinene, 3-carene, myrcene, limo-

nene and  $\gamma$ -terpinene), respectively. This finding indicated that the main emulsion components had more significant ( $p < 0.05$ ) effect on the equilibrium headspace concentration of the hydrophobic volatile flavour compounds (with high  $\log P$ ) compared to hydrophilic compounds (i.e. ethyl acetate and ethyl butyrate).

Fig. 1 exhibited how interaction effects of main emulsion components significantly ( $p < 0.05$ ) influenced the equilibrium headspace concentration of target volatile flavour compounds. As shown in Fig. 1, when all experimental variables were simultaneously altered, nonlinear relationships were significantly ( $p < 0.05$ ) fitted to the experimental data for describing the changes of the equilibrium headspace concentration as function of main emulsion components. Amongst all response variable effects, the interaction effect of xanthan gum and orange oil had the most significant ( $p < 0.05$ ) effect positive effect on the equilibrium headspace concentration of most of volatile flavour compounds except for ethyl butyrate,  $\gamma$ -terpinene and linalool (Table 2). The interaction effect of independent variables had the most and least

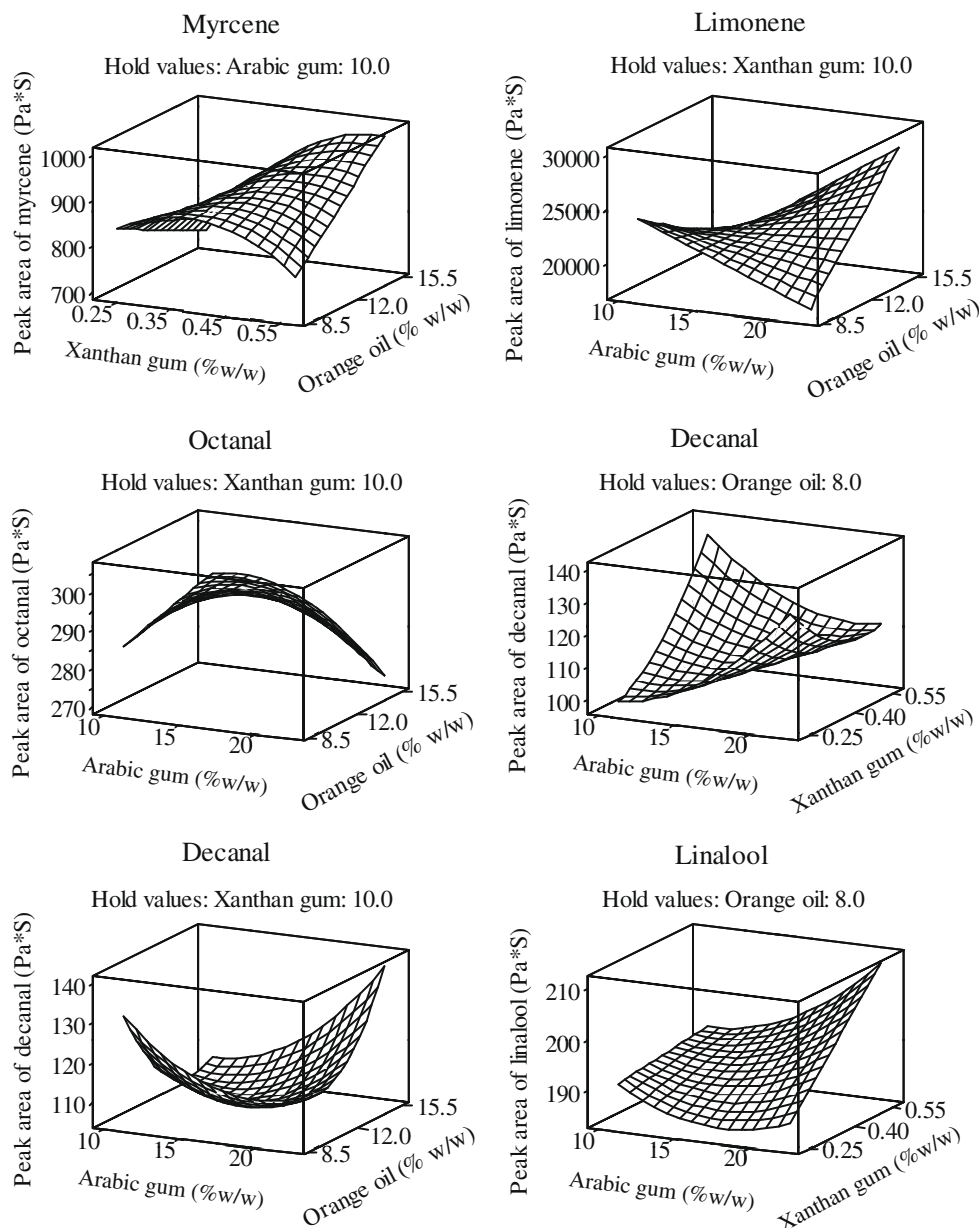
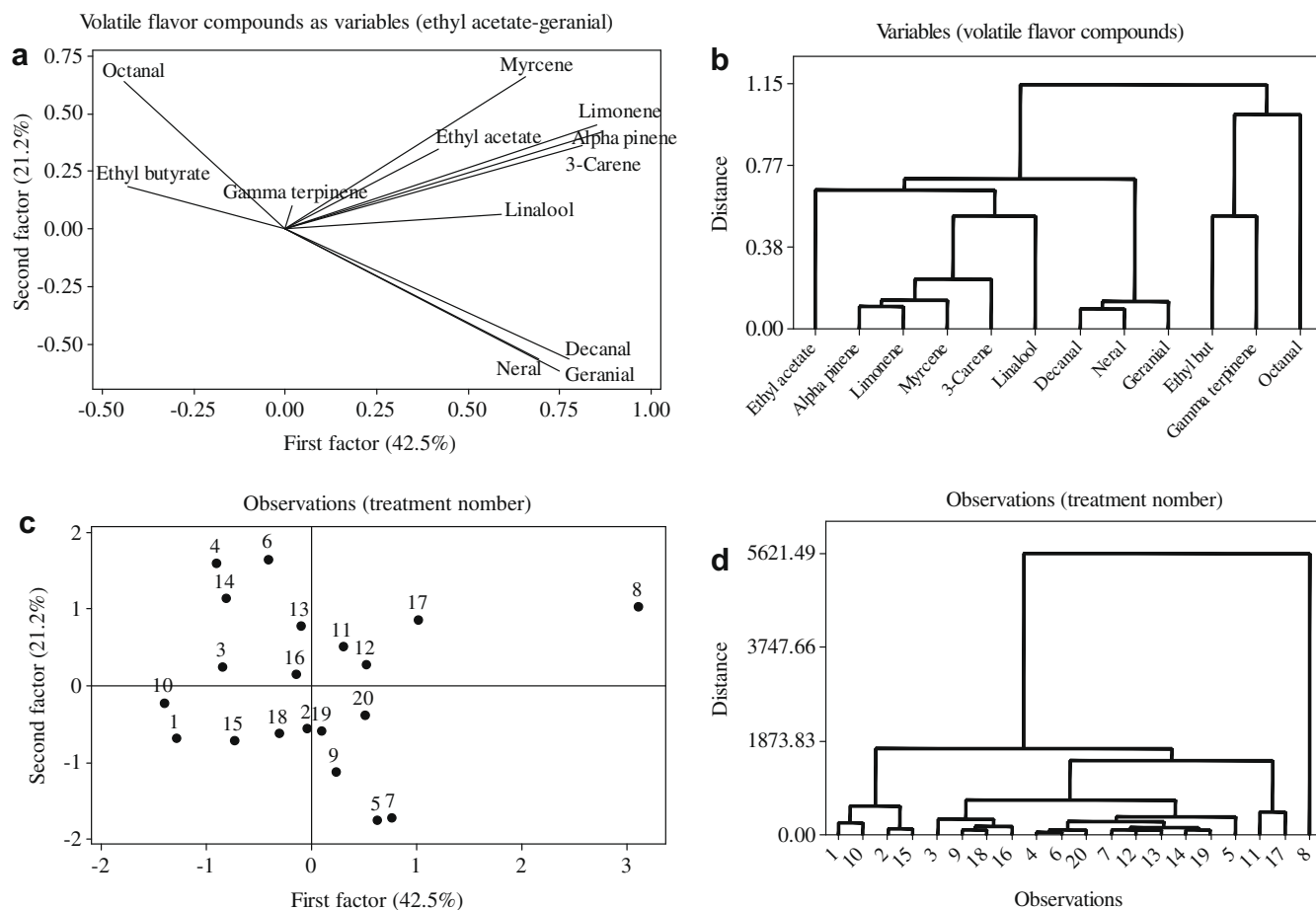


Fig. 1. Response surface plots for the response variables studied as a function of Arabic gum, xanthan gum and orange oil contents.



**Fig. 2.** PCA scores and cluster dendrogram of orange beverage emulsions; (a,b) PCA variable loadings of 13 orange volatile compounds and (c,d) PCA variable loadings of 20 orange beverage emulsions based on first and second principal components.

significant ( $p < 0.05$ ) effects on the equilibrium headspace concentration of linalool and  $\alpha$ -pinene respectively (Table 3). Except for ethyl butyrate,  $\gamma$ -terpinene, octanal, decanal, neral and geranial, the interaction effect of Arabic gum and orange oil had the significant ( $p < 0.05$ ) positive effect on the equilibrium headspace concentration of target volatile flavour compounds (Table 2).

### 3.5. Multivariate analysis

PCA score plots were used to determine whether twenty beverage emulsions containing various oil phase and emulsifier fraction content could be grouped into different classes (Fig. 2a and b). To focus on the differences amongst the orange beverage emulsions and target volatile compounds, cluster observation and cluster variable dendrograms were constructed using the nearest neighbour (Fig. 2c and d). The first two principal components explained 42.5% and 63.7% of the total variability, respectively. Despite first three principal components, which showed 79.7% of total variation, the remaining principal components accounted for very small proportion of the total variability and were probably unimportant (see Table 4).

Except for ethyl butyrate and octanal, the target volatile compounds could be classified in one group in PC1, because the coefficients of these volatile compounds were the same positive sign and not close to zero in PC 1 (Fig. 2a). Except for ethyl acetate, the volatile compounds directed in positive side of PC 1 showed high hydrophobicity level (or high  $\log P > 3$ ). On the other hand, the volatile flavour compounds with lower  $\log P (< 2.7)$  except for ethyl

acetate were located in the negative side of PC 1 (Fig. 2a). In most cases, the differentiation or closeness between the volatile flavour compounds directed in positive side of PC 1 was dependent on their chemical classes and magnitude of hydrophobicity or  $\log P$ . (see Table 5)

The second principal component (PC 2) accounted for 21.2% of the data variability could be thought as contrasting level of all monoterpene hydrocarbons (i.e.  $\alpha$ -pinene,  $\beta$ -pinene, 3-carene, myrcene, limonene and  $\gamma$ -terpinene), alcohol (i.e. linalool) and ester compounds (i.e. ethyl acetate and ethyl butyrate) with the same positive sign; whilst all aldehyde compounds except for octanal were placed in the negative effect of PC 2 (Fig. 2a). As compared to PC1, the differentiation in PC 2 appeared to be independent on the hydrophobicity level (or  $\log P$ ) of target volatile flavour compounds. The first nine volatile flavour compounds (except for decanal) in chromatogram were located in the positive side of PC 2 (Fig. 2a). The beverage emulsion no. 8 was mainly separated from the other samples based on the PCA scores. The beverage emulsion 8 had the highest positive loading score PCA plot because it showed the significant ( $p < 0.05$ ) highest total equilibrium headspace concentrations amongst all prepared beverage emulsion. The cluster dendrogram classified the samples 1, 10 and 2, 15 at close situated clusters because they showed the least equilibrium volatile headspace concentrations (Fig. 2c and d) (see Table 6).

The results obtained from the cluster analysis differentiated the beverage emulsions containing different emulsion compositions from each other (Fig. 2c, d). For instance, the beverage emulsions containing the same orange oil content but different content of

**Table 4**

Comparison between experimental and predicted values based on the final reduced models (validation procedure).

Run	Ethyl acetate <sup>a</sup>			$\alpha$ -Pinene <sup>a</sup>			Ethyl butyrate <sup>a</sup>			3-Carene <sup>a</sup>			Myrcene <sup>a</sup>		
	$Y_0$	$Y_i$	$Y_0 - Y_i$	$Y_0$	$Y_i$	$Y_0 - Y_i$	$Y_0$	$Y_i$	$Y_0 - Y_i$	$Y_0$	$Y_i$	$Y_0 - Y_i$	$Y_0$	$Y_i$	$Y_0 - Y_i$
1	5.0	4.8	0.2	66	65	1	355	342	13	40.0	39.1	0.9	800	794	6
2	7.0	7.3	-0.3	73	77	-4	332	326	6	50.0	51.1	-1.1	822	866	-44
3	6.8	6.9	-0.1	68	72	-4	352	359	-7	48.0	48.9	-0.9	814	834	-20
4	7.0	6.8	0.2	73	73	0	327	338	-11	46.0	46.3	-0.3	913	880	33
5	8.0	8.1	-0.1	77	79	-2	322	323	-1	46.0	46.3	-0.3	816	831	-16
6	7.0	6.8	0.2	83	73	10	338	338	0	48.0	46.3	1.7	921	880	41
7	6.0	6.2	-0.2	77	79	-2	321	318	3	46.5	47.4	-0.9	870	872	-2
8	8.8	8.7	0.1	104	102	2	328	340	-12	60.0	58.8	1.2	1000	987	13
9	7.0	7.0	-0.0	74	74	0	315	315	-0	44.5	44.6	-0.1	854	828	27
10	8.0	8.2	-0.2	67	70	-3	370	357	13	42.0	42.6	-0.6	769	782	-13
11	7.4	7.5	-0.1	78	81	-3	331	333	-2	49.0	48.3	0.7	919	915	4
12	8.0	7.5	0.5	88	81	7	330	333	-3	48.0	48.3	-0.3	887	915	-28
13	7.2	7.5	-0.3	82	78	4	365	367	-2	50.0	45.9	4.1	903	909	-6
14	8.0	7.5	0.5	78	78	0	382	367	15	41.0	45.9	-4.9	912	909	3
15	8.0	8.2	-0.2	73	72	1	379	373	6	43.5	43.1	0.4	799	791	8
16	8.0	7.5	0.5	76	74	2	364	375	-11	46.0	46.1	-0.1	873	830	43
17	9.0	9.0	0.0	89	86	3	365	369	-4	52.0	49.8	2.2	941	937	4
18	5.8	6.0	-0.2	72	71	1	359	365	-6	44.5	42.1	2.4	842	881	-39
19	7.5	7.5	-0.0	76	83	-7	357	359	-2	42.5	45.7	-3.2	841	865	-24
20	6.5	6.8	-0.3	82	85	-3	365	361	4	48.0	48.8	-0.8	902	891	11
Run	Limonene			$\gamma$ -Terpinene			Octanal			Decanal			Linalool		
	$Y_0$	$Y_i$	$Y_0 - Y_i$	$Y_0$	$Y_i$	$Y_0 - Y_i$	$Y_0$	$Y_i$	$Y_0 - Y_i$	$Y_0$	$Y_i$	$Y_0 - Y_i$	$Y_0$	$Y_i$	$Y_0 - Y_i$
1	19513	19461	52	267	259	8	300	303	-3	110	108	2	198	199	-1
2	20240	21508	-1268	292	312	-20	286	285	1	117	115	2	200	200	0
3	21127	21737	-610	285	282	3	294	294	0	106	106	0	195	196	-1
4	22639	21336	1303	259	273	-14	303	298	5	99	104	-5	190	192	-2
5	21903	22638	-735	297	289	8	271	275	-4	123	121	3	189	188	1
6	22593	21336	1257	288	273	15	300	298	2	102	104	-2	194	192	2
7	22382	22933	-551	271	278	-7	279	281	-2	127	130	-3	192	192	0
8	28068	27483	585	287	285	2	285	284	1	125	124	1	210	208	2
9	21413	21431	-18	243	255	-13	285	285	-0	118	119	-1	195	196	-1
10	19753	19829	-76	306	308	-2	288	290	-2	103	105	-2	189	188	0
11	23671	22919	752	264	269	-5	295	294	1	113	111	2	191	192	-1
12	22228	22919	-691	295	269	26	297	294	3	114	111	3	193	192	1
13	22314	22072	243	305	303	2	291	296	-5	109	107	2	190	190	0
14	22095	22072	24	303	303	-1	297	296	1	105	107	-2	189	190	-1
15	20337	20898	-561	293	303	-10	280	277	3	109	108	1	185	184	1
16	21552	21050	502	395	381	14	290	289	1	113	111	2	191	191	-0
17	24123	23275	848	307	302	5	287	290	-3	115	117	-2	194	196	-2
18	21491	20868	623	256	253	3	300	302	-2	118	116	2	197	195	2
19	22132	23093	-961	337	343	-6	290	287	3	115	117	-2	198	198	-0
20	22528	23245	-717	297	303	-6	282	281	1	118	119	-1	197	196	0
Run	Neral <sup>a</sup>			Geranial			Total flavor compounds with limonene <sup>a</sup>			Total flavour compounds without limonene <sup>a</sup>					
	$Y_0$	$Y_i$	$Y_0 - Y_i$	$Y_0$	$Y_i$	$Y_0 - Y_i$	$Y_0$	$Y_i$	$Y_0 - Y_i$	$Y_0$	$Y_i$	$Y_0 - Y_i$			
1	23.0	22.4	0.6	52	51.9	0.1	21729	21652	77	2216	2181	35			
2	24.0	23.8	0.2	51	48.6	2.4	22494	23838	-1344	2256	2321	-65			
3	22.0	22.1	-0.1	50	51.4	-1.4	23368	23993	-625	2241	2247	-6			
4	22.0	22.6	-0.6	42	45.6	-3.6	24920	23598	1322	2281	2282	-1			
5	27.5	27.0	0.5	56.5	53.3	3.2	24135	24908	-773	2232	2260	-28			
6	22.0	22.6	-0.613	45	45.6	-0.6	24941	23598	1343	2348	2282	66			
7	26.5	26.7	-0.2	61.5	61.8	-0.3	24660	25256	-596	2278	2312	-34			
8	27.0	26.9	0.1	62	63.6	-1.6	30564	29954	610	2496	2461	35			
9	25.5	25.7	-0.2	57	56.9	0.1	23630	23655	-25	2217	2215	3			
10	22.0	22.3	-0.3	51	53.6	-2.6	21967	22059	-92	2214	2220	-6			
11	24.0	24.2	-0.2	54	53.3	0.7	25996	25231	765	2325	2332	-7			
12	25.0	24.2	0.8	57	53.3	3.7	24570	25231	-661	2342	2332	10			
13	23.0	23.4	-0.4	52	50.8	1.2	24691	24432	259	2377	2381	-4			
14	22.0	23.4	-1.4	49.5	50.8	-1.3	24481	24432	49	2386	2381	5			
15	24.5	24.3	0.2	53.5	53.1	0.4	22585	23191	-606	2248	2233	15			
16	24.0	23.7	0.3	54.5	53.4	1.1	23987	23407	580	2435	2378	57			
17	25.0	24.0	1.0	55.5	56.4	-0.9	26563	25699	864	2440	2444	-4			
18	24.0	22.8	1.2	58.5	57.6	0.9	23767	23166	601	2276	2318	-42			
19	26.0	26.6	-0.6	56.5	57.6	-1.1	24478	25458	-980	2346	2384	-38			
20	25.0	25.4	-0.4	57	57.4	-0.4	24907	25674	-767	2379	2369	10			

 $Y_0$ : Experimental value;  $Y_i$ : predicted value;  $Y_0 - Y_i$ : residue value.<sup>a</sup> No significant ( $p > 0.05$ ) difference between experimental ( $Y_0$ ) and predicted value ( $Y_i$ ).



**Table 5**  
Comparison between experimental and predicted values in two predicted emulsion formulations.

Volatile flavor compound	Emulsion 1 <sup>a</sup>			Emulsion 2 <sup>a</sup>		
	Y <sub>0</sub>	Y <sub>i</sub>	Y <sub>0</sub> -Y <sub>i</sub>	Y <sub>0</sub>	Y <sub>i</sub>	Y <sub>0</sub> -Y <sub>i</sub>
Ethyl acetate	5.65	7.32	-1.67	12.18	9.98	2.20
α-Pinene	58.44	72.27	-13.83	101.45	107.85	-6.40
Ethyl butyrate	319.65	335.44	-15.79	348.26	363.71	-15.45
3-Carene	53.37	44.80	8.57	72.76	61.53	11.23
Myrcene	852.78	820.85	31.93	951.34	973.18	-21.84
Limonene	22878.00	21210.00	1668.00	26456.00	28890.00	-2434.00
γ-Terpinene	243.28	267.60	-24.32	326.98	326.39	0.59
Octanal	268.76	283.16	-14.40	262.13	280.59	-18.46
Decanal	121.48	112.96	8.52	139.65	126.73	12.92
Linalool	205.83	191.35	14.48	231.37	215.53	15.84
Neral	21.36	24.81	-3.45	31.31	27.76	3.55
Geranial	48.29	52.65	-4.36	73.87	67.44	6.43

Emulsion 1: emulsion with the least flavor release containing 18.56% (w/w) Arabic gum, 0.27% (w/w) xanthan gum and 10.61% (w/w) orange oil. Emulsion 2: emulsion with the highest flavor release composed of 22.22% (w/w) Arabic gum, 0.52% (w/w) xanthan gum and 14.21% (w/w) orange oil. Y<sub>0</sub>: experimental value; Y<sub>i</sub>: predicted value; Y<sub>0</sub>-Y<sub>i</sub>: residue value.

<sup>a</sup> No significant ( $p > 0.05$ ) difference between experimental (Y<sub>0</sub>) and predicted value (Y<sub>i</sub>).

emulsifier fractions (e.g. treatment no. 2, 5, 8 and 10) were classified in different PCA classes with relatively high distance (Fig. 2c, d). This observation highlighted the significant ( $p < 0.05$ ) effect of the emulsifier fraction on the equilibrium headspace concentration. This finding may be interpreted by the interaction between flavour molecules and emulsifier fraction adsorbed at the interfacial area, thus leading to the changes of the mass transport of volatile compounds through the liquid interfacial boundary layer. This interaction could also result in the physical entrapment of flavour molecules within the emulsion matrix because of several mechanisms such as adsorption, complexation leading to entrapment, encapsulation and hydrogen bonds (Carr et al., 1996). On the other hand, the orange beverage emulsions containing the same content of main emulsion components (no. 4, 6, 14 or 11, 12 and 13) were located at close situations in PCA plot (Fig. 2c, d).

**Table 6**  
Principle component analysis indicating eigenanalysis of the correlation matrix.

Eigenvalue	5.1009	2.5394	1.9179	0.8979	0.5223	0.3930
Proportion	0.425	0.212	0.160	0.075	0.044	0.033
Cumulative	0.425	0.637	0.797	0.871	0.915	0.948
Eigenvalue	0.2726	0.1581	0.0908	0.0539	0.0412	0.0121
Proportion	0.023	0.013	0.008	0.004	0.003	0.001
Cumulative	0.970	0.984	0.991	0.996	0.999	1.000
Variable	PC1	PC2	PC3	PC4	PC5	PC6
Ethyl ac	-0.186	-0.218	-0.496	-0.330	-0.135	-0.152
Alpha pi	-0.382	-0.262	-0.082	0.014	0.140	0.018
Ethyl bu	0.191	-0.116	-0.432	0.511	0.430	0.461
3-Carene	-0.359	-0.227	0.054	-0.060	-0.319	-0.395
Myrcene	-0.290	-0.413	0.068	-0.019	0.327	0.262
Limonene	-0.377	-0.282	0.036	-0.037	0.188	0.038
Gamma te	-0.008	-0.063	-0.590	0.336	-0.432	0.516
Octanal	0.194	-0.401	0.305	0.302	0.101	0.397
Decanal	-0.332	0.388	0.045	0.130	0.075	0.103
Linalool	-0.261	-0.041	0.314	0.560	-0.464	-0.234
Neral	-0.343	0.354	-0.075	-0.080	0.054	0.218
Geranial	-0.307	0.355	-0.063	0.288	0.336	0.033
Variable	PC7	PC8	PC9	PC10	PC11	PC12
Ethyl ac	-0.625	0.086	0.096	0.315	-0.119	-0.015
Alpha pi	-0.019	-0.046	-0.763	-0.141	0.234	0.314
Ethyl bu	0.077	-0.119	0.007	-0.075	-0.282	0.015
3-Carene	0.372	0.530	0.027	-0.194	-0.261	-0.172
Myrcene	0.295	-0.208	0.056	0.473	-0.072	-0.449
Limonene	-0.022	-0.193	0.611	-0.393	0.176	0.378
Gamma te	0.248	0.043	0.067	-0.064	0.099	0.014
Octanal	-0.384	0.396	-0.030	-0.121	-0.353	0.072
Decanal	0.135	0.134	0.061	0.459	-0.383	0.554
Linalool	-0.276	-0.359	0.012	0.159	0.079	-0.102
Neral	-0.166	-0.288	-0.115	-0.452	-0.508	-0.330
Geranial	-0.215	0.476	0.094	-0.001	0.450	-0.309

### 3.6. Optimisation procedure

The results obtained from numerical optimisation showed that the orange beverage emulsion containing 22.2% (w/w) Arabic gum, 0.52% (w/w) xanthan gum and 14.21% (w/w) orange oil provided the highest equilibrium headspace concentration of target volatile flavour compounds. On the other hand, the least equilibrium headspace concentration was obtained by the beverage emulsion containing 18.56% (w/w) Arabic gum, 0.27% (w/w) xanthan gum and 10.61% (w/w) orange oil. From the results obtained, the presence of sufficient amount of Arabic gum and xanthan gum at the o/w interface area decreased the mass transfer of target volatile flavour compounds from the liquid phase into the vapour phase, thereby reducing the equilibrium headspace concentration; whilst the presence of high concentrations of xanthan gum, Arabic gum and orange oil appeared to increase the equilibrium headspace concentration. The results indicated that the average droplet size increased with increasing the content of main emulsion components thereby reducing o/w interfacial area. Previous investigators (Seuvre, Espinosa Díaz, & Voilley, 2000) also demonstrated that interfacial interactions between surface active agents and aroma compounds decreased when surface active agents were present in excess, thus resulting in higher volatile release intensity.

### 3.7. Verification of the reduced models

The experimental values were statistically compared with those predicted values in order to verify the validity of the final reduced. No significant ( $p > 0.05$ ) difference between the experimental and predicted values was observed (data not shown). The results also showed a close correspondence between those values. Thus, the experimental values were found to be in agreement with the predicted ones. The recommended beverage emulsions were practically prepared and then evaluated in order to compare their overall equilibrium headspace concentration with the predicted ones. Under the corresponding condition, the experimental values for the equilibrium headspace concentration of two orange beverage emulsions were close to the predicted ones (data not shown). Closeness between those values indicated the accuracy of the response regression equations relating the responses to independent variables.

## 4. Conclusions

The present study demonstrated that the equilibrium headspace concentration of target volatile flavour compounds from

the orange beverage emulsion could be modified by the changes of the emulsion composition and structure. Amongst the volatile flavour compounds, the independent variables exhibited the most significant ( $p < 0.05$ ) effect on the equilibrium headspace concentration of aldehyde compounds studied (i.e. octanal, decanal, neral and geraniol), alcohol (i.e. linalool) and monoterpene hydrocarbons (i.e.  $\alpha$ -pinene,  $\beta$ -pinene, 3-carene, myrcene, limonene and  $\gamma$ -terpinene) rather than hydrophilic compounds (i.e. ethyl acetate and ethyl butyrate). The equilibrium headspace concentration of target orange flavour compounds released from the beverage emulsion was significantly ( $p < 0.05$ ) governed by their hydrophobicity and chemical classes. Multivariate analysis differentiated the orange beverage emulsions containing the same orange oil content, but different concentration of hydrocolloid fractions. Whereas the beverage emulsions containing the same hydrocolloid and orange oil contents (i.e. centre points) were classified into the similar groups. This finding highlighted the influence of interaction between volatile flavour compounds and emulsifier fraction affecting the equilibrium headspace concentration of target volatile compounds.

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