2012-08-31

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Recommended Citation

M. Traynor, R. Burke, N. Brunton & C. Barry-Ryan (2012) Response Surface Methodology Guided Release of Two Acetate Volatiles From an Oil-in-Water Emulsion, *Journal of Culinary Science & Technology*, 10:3, 223-238, DOI: 10.1080/15428052.2012.706137

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ABSTRACT

The optimisation of the volatile release of two commonly used flavour compounds (Isoamyl acetate and Furfuryl acetate) from a food emulsion model system was evaluated using Response Surface Methodology (RSM). 27 random order settings were established using a Central Composite Faced Centered experimental design (CCF). The main and combined effects of four independent variables; concentration of Isoamyl acetate (50-90 ppm), Furfuryl acetate (20-30 ppm), salt (NaCl) (0.1-2 %) and pH (5-7) on the responses were examined. The main objective of the present study was to determine the optimal concentration level of the four variables leading to optimal release of the volatile compounds. Quantitative measurements were conducted using solid-phase microextraction (SPME) coupled with gas chromatography mass spectrometry (GC-MS). Salt concentration and Isoamyl acetate concentration were found to have significant positive effects ($p < 0.001$) on the release of Isoamyl acetate. The optimisation procedure indicated that the optimal conditions leading to the desirable volatile release was Isoamyl acetate 90 ppm, Furfuryl acetate 30 ppm, salt 2 % w/v and pH 6.0176. The findings of this study can enable chefs and food manufacturers to optimise conditions for maximum flavour release from food emulsion products.

KEYWORDS Solid-phase microextraction, gas chromatography, Isoamyl acetate; Furfuryl acetate; response surface methodology; food emulsion.

INTRODUCTION

In recent times, chefs, culinary experts and food processors have become interested in the intelligent design of foods with optimal flavour characteristics (Roberts & Taylor, 2000). Flavour is considered as one of the most important attributes determining the acceptance of food by consumers (Clarke, 1998). Therefore, information on the effect of the characteristics of food matrices with respect to flavour volatile release is vital. The production of foods/dishes in a kitchen or in a food manufacturing plant involves manipulation of the many physiochemical characteristics of the product which can influence the release of volatile molecules during gustation. For example, many foods contain significant levels of added sodium chloride (NaCl) both to deliver flavour and act as a preservative (Mitchell *et al.*, 2011). Sodium chloride concentrations will have a significant influence on the ionic strength of a food which will in turn influence the solubility of flavour compounds depending on their hydrophobicity (Rabe *et al.*, 2003a). In fact, the practice of “salting out” is a well known technique for increasing headspace concentrations of hydrophobic volatile molecules prior to separation and quantification by headspace gas chromatography (Guichard, 2002; Flores *et al.*, 2007; Pérez-Juan *et al.*, 2007).

The pH of a food matrix can have a strong influence on the ionic state of volatile aroma compounds contained therein and therefore influence their concentration during gustation (Reineccius, 2005). The physiochemical characteristics of small molecules such as flavour

Most foods are highly complex systems, however, many can be characterised as emulsions, i.e. dispersed systems of oil and aqueous phases (McClements, 2005). Emulsions are an important class of food colloids and a wide range of products can be classed as emulsions or consist of emulsions such as salad dressings, ice cream, cream liqueurs or soft drinks (Schramm, 2005; Guzey & McClements, 2006). The solubility of flavour compounds in the phases of the emulsions has a determinantal role in the behaviour of the flavours in the matrix and will have an over-riding influence on their sensory perception (Landy *et al.*, 1998).

Flavour compounds are often added to the formulations of complex foods to impart characteristic flavours. For example, Isoamyl acetate is one of the most employed flavour compounds in foods (74,000 kg per annum) because of its characteristic banana flavour (Yilmaztekin *et al.*, 2008). Also, Furfuryl acetate is another flavour compound frequently used in foods as a flavourant (Burdock, 2010). Even in a simple model food system a vast array of factors can influence volatile flavour release therefore many studies employ the use of response surface methodology to reduce the number of experiments required to optimise extraction conditions (Mirhosseini & Tan, 2009; Cheong *et al.*, 2011; Ribeiro *et al.*, 2011).

In the present study, response surface methodology (RSM) was used to (1) model and optimise the conditions (pH 5-7 and salt concentration (NaCl) 0.01 % - 2 %) leading to maximum flavour release, and (2) to study the main and interactive effects on banana volatile concentration (Isoamyl acetate 50-100 ppm and Furfuryl acetate 20-30 ppm). Concentration

Isoamyl acetate and Furfuryl acetate were purchased from Sigma Aldrich (Dublin, Ireland). Sunflower oil (Basso Fedele and Figli, Avellino, Italy) and soy lecithin (Kelkin, Dublin, Ireland) were purchased in a local supermarket. Xanthan gum was kindly donated by Chemcolloids Ltd. (Cheshire, UK). Citric acid and hydrogen sodium carbonate, SPME fibre, 20 mL glass vial and Teflon coated rubber septa and aluminium screw caps were purchased from Sigma Aldrich (Dublin, Ireland.)

Preparation of Emulsions

The surfactant solutions were prepared by dispersing the soy lecithin and xanthan gum in distilled water and mixing for 1.5 hours at 60 °C using a magnetic stirring bar and magnetic stirrer hotplate (Stuart CB162, Bibby-scientific, Staffordshire, UK). The lipid phase was then heated to 60 °C and the flavour compounds were then added. The lipid phase was then slowly added to the aqueous phase while stirring. This pre-emulsion was allowed to mix for a further 5 minutes before being homogenised with a high shear blender (Silverson L4R, Silverson Machines Ltd., Chesham, UK) for 3 minutes at 8,000 rpm. NaCl was added to the emulsion and was allowed to dissolve under gentle stirring for 1-2 min. The pH was adjusted using the 0.1 M citric acid and 0.5 M sodium hydrogen carbonate, the pH was monitored using a pH meter (Thermo scientific, Orion 2 Star, Essex, UK).

mL aliquots of the emulsion into a 20 mL headspace glass vial (O.D 2.25 mm x H 75 mm) which was sealed with a stainless steel magnetic screw cap fitted with a Polytetrafluoroethylene (PTFE)/silicone septum (septum thickness 1.3 mm). A PTFE coated micro stirring bar (1/2 in. x 3/8 in.) was used to simulate shear stress in the mouth. A shear rate of approximately 150 s^{-1} was achieved by stirring at 450 rpm (Rabe *et al.*, 2003b). Vials were placed in a water bath set at 40 °C and allowed to equilibrate for 5 min. A SPME fibre coated with 65 µm Polydimethylsiloxane-Divinylbenzene (PDMS/DVB) was manually inserted into the headspace of the vials for adsorption. Subsequently the SPME fibre was removed from the headspace and desorbed at 250 °C in the GC injection port for 3 min. The fibre was cleaned after every two extractions to ensure no carry-over between samples by soaking it in acetone for at least 15 minutes and then rinsing with 40 % ethanol before reuse (Lay-Keow, 1998).

GC Conditions

Analysis of the volatile compounds absorbed on the fibre was carried out using a Varian 3800 GC coupled to a Varian Saturn 2000 ion trap mass spectrometer (Varian Chromatography Systems, Walnut Creek, CA, USA). Separation of the volatiles was accomplished on a ZB-wax column (ZB-5MS- 15 m x 0.25 mm i.d., 0.25 µm film, Torrance, CA, USA). Helium, at a flow rate of 1 mL/min was used as the carrier gas. Thermal desorption of the compounds took place in the GC injection port (1079 Programmable

were recorded after electron impact ionization under EI auto mode. Peak areas were analysed and quantified using the Varian star chromatography workstation software (v 5.0; Varian Chromatography Systems). All results were expressed as the mean values of three independent trials.

Isoamyl acetate and Furfuryl acetate were initially identified using a Varian 450-GC equipped with a Varian 320-Ms triple quadrupole (Varian Chromatography Systems, Walnut Creek, CA, USA) and CombiPal Autosampler (CTC Analytics). Compounds were also identified by use of authenticated standards and by matching mass spectra with the data stored in the NIST library of standard compounds. The data reported was the mean of 3 extraction replicates for each individual peak in the total ion chromatogram (TIC). Compounds were quantified by reference to external calibration curves constructed using the same authenticated standards and expressed as parts per million. The standard curve had an R^2 value of 0.996 and 0.976 for the Isoamyl acetate and Furfuryl acetate respectively.

Experimental Design

In this study, a RSM approach was employed to (1) study the main effect and combined effect of these independent variables on response variables, (2) create empirical models between the variables and (3) optimise the physiochemical conditions for maximum volatile release in terms of the response variables studied. The effect of four independent variables

namely, x_1 (Isoamyl acetate concentration 50-900 ppm), x_2 (Furfuryl acetate concentration 20-

The volatile release of each flavour compound was expressed by the peak area recorded by using GCMS. The concentration of each volatile in the headspace above the emulsion was considered as response variables in the present study. This was calculated by comparing the response for both the volatiles to that of a standard calibration curve. Table 1 illustrates the independent variables, dependent variables and the experimental design factor setting for the CCF design including the values corresponding to the levels of factors and treatments, assuming four factors, each with low, medium and high settings.

Statistical Analysis

The effects of the independent variables on the volatile flavour release of Isoamyl acetate (Y_1) and Furfuryl acetate (Y_2) from an oil in water emulsion and their interaction were analyzed using polynomial regression analysis and analysis of variance (ANOVA). A second order polynomial equation for dependent variables was established to fit the experimental data. The proposed generalised second order polynomial equation is given as

$$Y_i = \hat{a}_0 + \hat{a}_1x_1 + \hat{a}_2x_2 + \hat{a}_3x_3 + \hat{a}_4x_4 + \hat{a}_{11}x_1^2 + \hat{a}_{22}x_2^2 + \hat{a}_{33}x_3^2 + \hat{a}_{44}x_4^2 + \hat{a}_{12}x_1x_2 + \hat{a}_{13}x_1x_3 + \hat{a}_{14}x_1x_4 + \hat{a}_{23}x_2x_3 + \hat{a}_{24}x_2x_4 + \hat{a}_{34}x_3x_4 \quad (1)$$

Where Y_1 is the predicted response, x_1 , x_2 , x_3 and x_4 are the independent linear variables, x_1^2 , x_2^2 , x_3^2 and x_4^2 are the independent quadratic variables and x_1x_2 , x_2x_3 , x_2x_4 and x_3x_4 are the independent interaction variables. In the model, $\hat{a}_0$ is the intercept term, $\hat{a}_1$, $\hat{a}_2$, $\hat{a}_3$ and $\hat{a}_4$ are the regression coefficients for the linear effect, $\hat{a}_{11}$, $\hat{a}_{22}$, $\hat{a}_{33}$ and $\hat{a}_{44}$ are quadratic

and data was reported as the mean $\pm$ standard error (SE). To determine the significance of a model parameter, the *t*-student test was used. Differences were considered to be statistically significant at $p = 0.05$.

Validation of Optimal Conditions

The adequacy of response surface models for predicting the optimum response values was verified by conducting experiments under the recommended optimum conditions. The experimental predicted values of the responses were compared in order to check the validity of the models.

Table 1 Central Composite Faced design: independent variables (x_i) and response variables(Y_j)

Run	Independent variables				Response Variables			
	Isoamyl acetate (ppm) (x_1)	Furfuryl acetate (ppm) (x_2)	pH (x_3)	Salt (w/v%) (x_4)	Isoamyl acetate (ppm) (Y_1)	RSD %	Furfuryl acetate (ppm) (Y_2)	RSD%
1	50	20	5	0.1	150.203	7.54	136.656	10.78
2	90	20	5	0.1	284.492	4.99	246.29	6.12
3	50	30	5	0.1	106.706	3.80	242.59	6.10
4	90	30	5	0.1	96.766	17.96	81.099	29.95
5	50	20	7	0.1	73.21	7.44	316.638	14.01
6	90	20	7	0.1	245.249	7.21	174.6	9.11
7	50	30	7	0.1	20.752	2.34	40.872	9.61
8	90	30	7	0.1	92.950	0.86	77.23	1.45
9	50	20	5	2	110.913	1.40	276.907	2.22
10	90	20	5	2	277.857	4.57	207.025	5.64
11	50	30	5	2	140.771	4.07	262.064	5.94
12	90	30	5	2	269.778	5.97	281.686	7.40
13	50	20	7	2	124.429	2.31	168.838	3.50
14	90	20	7	2	364.551	3.55	133.485	4.17
15	50	30	7	2	40.416	13.82	78.382	35.93
16	90	30	7	2	309.311	6.52	253.627	7.88
17	50	25	6	1.05	24.118	7.88	48.0824	29.00
18	90	25	6	1.05	287.81	2.27	241.216	2.78
19	70	20	6	1.05	193.997	21.54	101.409	28.72
20	70	30	6	1.05	197.999	7.19	317.921	9.54
21	70	25	5	1.05	144.283	6.77	192.837	9.80
22	70	25	7	1.05	56.220	0.10	54.711	0.21

RESULTS

Fitting the Response Surface Models and Repeatability

Regression analysis was carried out to fit mathematical models to the experimental data. The regression coefficients and analysis of variance of the coded independent variables are presented in Table 2. The resulting regression coefficients for the coded dependent variables showed that the regression model for Isoamyl acetate (Y_1) had a statistically good fit. The R^2 statistic indicated that the response surface model accounted for 90 % of variation in the Isoamyl acetate response (Y_1), which is above the minimum recommended R^2 value (0.80) (Joglekar & May, 1987). For the Furfuryl acetate response (Y_2), only 44 % of the variation could be explained by the response surface model, therefore having a statistically poor fit and poor prediction capabilities. Significant ($p < 0.001$) regression P -value and a non-significant ($p > 0.05$) lack of fit P -value indicated that the mathematical model fitted well to the experimental data for Isoamyl acetate (Y_1) (Bezerra *et al.*, 2008). Although the lack of fit P -value was non-significant ($p > 0.05$) for Furfuryl acetate response (Y_2), the regression P -value was also non-significant ($p > 0.05$), thus the mathematical model might require slight improvements.

The repeatability was determined to check the precision of the method. The repeatability of the experimental procedure was evaluated by calculating the relative standard deviation percentage (RSD %) of three replicates for each experimental condition (Table 1). The average RSD % values for the response were significantly above the acceptable level of 5%

Table 2 Regression coefficients and analysis of variance of coded dependent variables

Regression coefficients	Isoamyl acetate (Y_1)	Furfuryl acetate (Y_2)
β_0	154.777***	156.99
β_1	79.8471 ***	6.95713
β_2	-30.5251*	-7.0209
β_3	-14.1489	-34.9317
β_4	36.3422***	27.0138
β_1^2	14.1938	-3.93245
β_2^2	54.2279	61.0833
β_3^2	-41.519	-24.8076
β_4^2	-14.0331	-4.26023
β_{12}	-15.8271	12.9608
β_{13}	20.8096	8.5205
β_{14}	27.2739*	15.448
β_{23}	-8.41039	-21.5007
β_{24}	19.9065	32.6185
β_{34}	15.3371	-18.5034
R^2	0.902	0.437
Regression (P -value)	0.00***	0.768
Lack of fit (P -value)	0.251	0.36

β_i : estimated regression for the main linear effects.

β_i^j : the estimated regression coefficients for the quadratic effects.

β_{ii} : the estimated regression coefficients for the interaction effects.

* Significant (p 0.05), ** Significant (p 0.01), *** Significant (p 0.001)

importance of the different regression coefficients, and provides a visual indication of whether a variable has a positive or negative influence on the response.

Figure 1 Main effects and significant parameters on the recovery of Isoamyl acetate from emulsion headspace

^a Significant (p 0.05), ^b Significant (p 0.001)

Isoamyl acetate concentration and salt concentration (x_{14}) on the Isoamyl acetate response (Y_1). With regards to the effect of Isoamyl acetate concentration on the release of Furfuryl acetate (Y_2) from the emulsion, no significant ($p > 0.05$) effects were found.

The regression results demonstrate that Furfuryl acetate concentration (x_2) had a significant effect ($p < 0.05$) on the Isoamyl acetate response (Y_1). Moreover, this effect of Furfuryl acetate on the release of Isoamyl acetate in to the headspace of the emulsion proved to be a negative effect. No significant ($p > 0.05$) effects were found for Furfuryl acetate concentration on the release of Furfuryl acetate (Y_2) from the emulsion. In addition, the pH (x_3) proved to have no significant influence ($p > 0.05$) on Isoamyl acetate (Y_1) or Furfuryl acetate (Y_2) release from the emulsion. Salt concentration (x_4), had a significantly ($p < 0.001$) positive effect on the volatile release of Isoamyl acetate (Y_1) (Figure 3).

Validation of Optimal Conditions

The optimal conditions for the targeted responses were generated by the Modde 5.0 software (Table 3). At optimal conditions (Isoamyl acetate 90 ppm (x_1), Furfuryl acetate 30 ppm (x_2), salt 2 % w/v (x_3) and pH 6.018 (x_4)), the predicted values were 326.41 ppm and 811.34 ppm for Isoamyl acetate (Y_1) and Furfuryl acetate (Y_2) release from the emulsion respectively. HS-SPME GCMS analysis was carried out at the optimal conditions to verify the model. At optimal conditions, the product contained Isoamyl acetate (273.75 ppm) (Y_1) and Furfuryl acetate (593.58 ppm) (Y_2), which was within the error ranges.

Figure 3 Response surface plot for effect of salt concentration % on Isoamyl acetate response

Table 3 List of predicted optimal conditions generated by Modde 5.0 RSM software

Isoamyl acetate (ppm) (x_1)	Furfuryl acetate (ppm) (x_2)	pH (x_3)	Salt (w/v%) (x_4)	Isoamyl acetate response (ppm) (Y_1)	Furfuryl acetate response (ppm) (Y_2)
88.7161	20	5.564	2	348.817	619.021
89.8708	20	5.530	2	354.724	618.816
90	30	6.253	2	326.96	764.177
*90	30	6.017	2	326.409	811.344
89.1528	20	5.540	2	350.355	619.016
89.4616	20	5.532	2	351.955	618.942
88.7752	20	5.558	2	348.871	619.04
90	30	6.163	2	327.295	783.01

*optimal condition selected by Modde 5.0 software from the list of generated optimal conditions

DISCUSSION

Justification of concentration ranges was based on the Flavour and Extract Manufacturers Association (FEMA) reported uses in literature (Burdock, 2010) and from preliminary sensory analysis, where differences in minimum and maximum concentrations of Furfuryl acetate were successfully identified by a panel (data not shown). Besides showing the optimal conditions for Isoamyl acetate recovery, the mathematical model identified and described significant effects of the independent variables and some interesting interactions between the variables on the Isoamyl acetate response. These interactions are substantial and a ‘one variable at a time’ approach may have proven too complicated for optimisation.

concentration range of the Furfuryl acetate (20-30 ppm) being quite narrow in comparison to Isoamyl acetate (50-90 ppm). Additionally, the contour plots for Furfuryl acetate release (Figure 4) shows a 'saddle point', which is a stationary point of neither a maximum nor minimum response (Myers *et al.*, 2009). This indicates that the optimum conditions for Furfuryl acetate release from the emulsion lie outside the experimental range investigated in this study.

Figure 4 Contour plot of Furfuryl acetate release from emulsion as a function of Furfuryl acetate concentration and pH

effect that Furfuryl acetate concentration had on the Isoamyl acetate volatile response could be a result of competition between the volatiles for space on the SPME fibre. It was found in previous studies (Matich *et al.*, 1996; Murray, 2001; Howard *et al.*, 2005), that volatile compounds exhibited competition for extractions sites on the SPME fibres. Higher molecular weight compounds can have the tendency to displace those with lower molecular weights, thereby causing inaccuracies in the relative amounts of analytes present, especially with fibres coated with PDMS. This may explain why an increase in concentration of the higher molecular weight compound Furfuryl acetate resulted in a decrease in the lower molecular weight compound Isoamyl acetate being absorbed onto the SPME fibre.

Furthermore, the possible competition for space on the fibre between the volatiles may have resulted in a “knock on” effect on the other variables, thus masking any additional significant effects on the responses. A possible solution to this may involve reducing the concentrations of both volatiles to a level that will result in competition for space on the fibre between the volatiles. However, the use of a concentration range below 20 ppm for Furfuryl acetate may have provided little useful information with regard to sensory properties of the emulsion.

Similarly, the lack of a significant effect ($p > 0.05$) of pH on the responses may be due to the narrow range of pH selected. This experimental range was selected to fit in with the context of the work being undertaken. Food products such as cream sauces and dressings would be expected to have pH <http://www.pdf4free.com> would be considered tolerable for

The perception of flavour and aroma volatiles in foods is influenced by the solubility of volatile compounds, which subsequently can be affected by the level of salt present in a food (Mitchell *et al.*, 2011). Salts are often added to aqueous samples to increase the concentrations of the aroma compounds in the vapour phase (Guichard, 2002). The addition of salt to foodstuffs results in the decreased availability of water molecules for the solubilisation of flavour compounds, thus, an increase in flavour release from the food product into its headspace, consequently an increase in perceived flavour concentration during consumption (Rabe *et al.*, 2003a; Flores *et al.*, 2007).

From an analytical point of view, dissolution of salt into the sample matrix is a simple way to enhance the partition coefficient into the headspace of the volatile compounds due to the 'salting out' effect in headspace analysis (Steffen & Pawliszyn, 1996). According to the results, salt concentration had a pronounced effect on the release of Isoamyl acetate from the emulsion. An increase in salt led to an increase in overall extraction yield of Isoamyl acetate from the emulsion (Figure 3). It would be expected that the addition of salt to the emulsion, increased the ionic strength of the solution, which subsequently decreased the solubility of the hydrophobic compounds in the aqueous phase, thereby increasing the partition coefficient of the volatile compounds (Rocha *et al.*, 2001).

Similar observations were also reported in previous studies (Mirhosseini *et al.*, 2007; Cheong *et al.*, 2010; Cheong *et al.*, 2011), where extraction efficiency increased with addition of salt. Mirhosseini *et al* (2007) <http://www.pdf4free.com> peak areas improved by 47 % to

effect salt on the extraction efficiency of volatiles from a matrix from a purely technical point of view, with a goal for extracting volatiles qualitatively, hence, much higher levels of salt were investigated (0-30 % w/v). In the present study, the model system was based around common food emulsions, such as cream sauces and dressing, therefore, human consumption was considered, and so salt content in the relatively narrow range of 0.1-2 % was investigated. The use of concentration above this level would have made the emulsion unacceptable from a sensory and product development point of view.

Modifying food flavour is a long standing practice, chefs, culinarians and food manufacturers aim to produce food products that are flavoursome and that maximise the enjoyment provided through the sensory experience for consumers. The way in which flavour components modify the perceived flavour of food has advanced from a stage of simply awareness of the situation, to an understanding of the interactions involved at a qualitative and quantitative level (Taylor & Hort, 2007). From a practical point of view, optimising the release from the food matrix of two flavour compounds that are strongly associated with positive fruity notes in foods can be a method of enhancing flavour perception. The perception of aroma and flavour is dependent up on the concentration and odour threshold of the volatile compounds present in the food (Guichard, 2002). Thus, finding the optimum concentration of volatile compounds for maximum flavour release is a crucial step in the development of flavoursome food emulsions, which are an important class of food colloids.

when creating food emulsions such as cream sauces and dressings etc. The main effects of Isoamyl acetate concentration and salt concentration should be considered as critical factors when studying the release of Isoamyl acetate from an oil in water emulsion.

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