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Feasibility and application of solvent assisted flavour evaporation and standard addition method to quantify the aroma compounds in flavoured baked matrices

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Abstract

In order to determine the feasibility of the solvent assisted flavour evaporation extraction (SAFE) technique and the standard addition method to quantify the aroma compounds retained in baked matrices after processing, three doughs that mainly vary in one ingredient, M1 (with eggs and palm oil), M2 (without eggs but with palm oil) and M3 (without eggs and without palm oil) were flavoured with an aromatic formulation of 19 different aroma compounds in propylene glycol that is responsible for a *viennoiserie* aromatic note. From the 19 aroma compounds added, 12 of them were quantified, so was also the propylene glycol. After the application of standard addition method 36 linear regression models were obtained and 28 of them showed good r^2 values and allowed quantification. The results from the comparison of the slopes (95%) showed significant differences of the aroma compounds depending on matrix composition, which influences the degree of interaction of the aroma compounds in the matrix and the losses of the aroma during processing. The percentage of retention of the aroma compounds after processing was lower in M3 than in matrices M1 and M2.

Keywords: Solvent assisted flavour evaporation technique; Standard addition method; Flavoured baked matrices; Aroma retention

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1. Introduction

Food flavouring is widely used in baked products to reinforce or to generate a desired aromatic note. Nevertheless in the initial elaboration steps (incorporation of the aroma in the dough, kneading, etc.) and mainly during baking significant losses of the added aroma compounds will occur (Brauss, Balders, Linforth, Avison, & Taylor, 1999; De Roos & Mansencal, 2003; Heiderich & Reineccius, 2001; Reineccius & Whorton, 1990). Flavour loss during baking can be mainly explained by the volatility of the flavour compounds in the matrix and the amount of water loss during the heating process (De Roos & Graf, 1995; Reineccius & Whorton, 1990). As most of the flavour compounds are hydrophobic, fat content highly influences flavour loss (Brauss et al., 1999; Clawson, Linforth, Ingham, & Taylor, 1996; De Roos & Mansencal, 2003; De Roos, 2003; Reineccius & Whorton, 1990).

In order to quantify the aroma compounds added to the baked cereal matrices or the compounds generated during baking, many different techniques for the isolation of aroma compounds have been proposed, but the most widely used are direct extraction with organic solvents (DE) (Brauss et al., 1999; De Roos & Graf, 1995; De Roos & Mansencal, 2003) and simultaneous steam distillation extraction (SDE) (Kirchhoff & Schieberle, 2002; Whorton & Reineccius, 1989; Zehentbauer & Grosch, 1998). Nevertheless, some drawbacks have been shown. With DE there could be significant losses of the most volatile compounds during the evaporation of the solvent (Prost, Lee, Giampaoli, & Richard, 1993), but also the extraction of non-volatile compounds could interfere with the analysis, and other

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clean-up steps will be necessary to remove them (De Roos & Mansencal, 2003; Kirchhoff & Schieberle, 2002). SDE has been shown to be less reliable in the extraction of the most polar volatile compounds (Prost et al., 1993). Also the elevated temperatures applied during distillation may lead to artefact formation and in matrices in which sugar and amino acids are present, Maillard or Strecker reactions could be responsible for the formation of new aroma compounds or the transformation of the endogenous and/or exogenous aroma compounds (Engel, Bahr, & Schieberle, 1999).

Heiderich and Reineccius (2001) have employed direct thermal desorption (DTD) to extract the aroma compounds from flavoured cookies. They have shown some advantages of this technique, which is rapid, repeatable and does not involve any sample preparation. Nevertheless, they have indicated that due to the low sensitivity of the technique the samples must have a high concentration of aroma compounds, be uncommon in flavoured commercial products, and have low moisture to avoid ice formation in the thermal desorber module.

Solvent assisted flavour evaporation technique (SAFE) described by Engel et al. (1999) allows careful isolation of volatile compounds from strawberries and tomato pulps based on their distillation under vacuum conditions. These authors have shown the feasibility of this technique for the isolation of volatile compounds from synthetic solutions, also when fatty materials are added, and for the extraction of furaneol from food matrices such as strawberry and tomato pulps. However, it has not been applied to any other foodstuffs.

Although direct calibration method has been largely employed for quantifying volatile compounds in food products, it has been shown that the different extraction coefficients of the aroma compounds and the interactions with the matrix can affect the quantification (Guichard, 1988). To try to solve these problems, other quantification methods such as stable isotope dilution assays have been successfully used to quantify some of the most important odorant compounds in bread after solvent (Schieberle & Grosch, 1987; Schieberle & Grosh, 1991) or headspace extraction (Zehentbauer & Grosch, 1997). The main advantages of this method are its sensitivity and repeatability; nevertheless it requires the availability of deuterated counterparts of all the compounds, which can make this technique arduous and expensive.

The quantification by the standard addition method also considers the interactions between the aroma compounds and the food product since it consists of the addition of known amounts of standard compounds directly to the samples and in the measurement of the solvent or headspace extract. The concentration of the standard in the sample is obtained by extrapolation in the standard addition graph. Although this method has been applied to quantify volatiles produced in some foodstuffs such as apricots (Guichard, 1988), cheese (Pinho, Ferreira, & Ferreira, 2002; Verzera, Ziino, Condurso, Romeo, & Zappalà, 2004), orange juice (Lum, Wong, & Lee, 1989), pasteurised milk and fermented milk (Imhof & Bosset, 1994), it has, however, not been employed to quantify the aroma compounds in flavoured baked cereal matrices.

The aim of this work is to determine the feasibility of using the SAFE extraction and the standard addition method to quantify the aroma compounds retained after processing in flavoured baked matrices when their composition changes and the application of this method to determine the retention of aroma compounds in the matrices after processing.

2. Materials and methods

2.1. Formulation of the matrices

Ingredients listed in Table 1 were purchased at a local market and were used to make a sponge cake dough (M1 dough) and two other simplified doughs, M2 (without eggs) and M3 (without eggs and without palm oil).

2.2. Dough flavouring

Flavouring was carried out using 19 aroma compounds responsible for a *viennoiserie* aromatic note solubilised in propylene glycol. Sigma Aldrich (I'lle d'Abeau, France) supplied all the aroma compounds for this mixture. The concentration of each compound in the mixture and various physico-chemical data are listed in Table 2.

2.3. Matrix preparation

2.3.1. Dough preparation

To obtain the M1 dough; eggs, sucrose and salt were mixed together with a household electric mixer in a water bath at 50 °C for 5 min. The mixture was removed from the water bath and after 1 min of rest was mixed again for 2 min. Flour was then added little by little, and gently incorporated into the foam with a wooden spoon. The aroma mixture was added to the melted palm oil and then immediately to the dough. For the elaboration of doughs M2 and M3 the ingredients were mixed without the water bath during the same time than that for the elaboration of M1 dough. For M3, the incorporation of the aroma mixture was added directly into the dough. In the three

Table 1
Formulation of the matrices

Ingredients (g/100 g dough)	M1-matrix	M2-matrix	M3-matrix
Wheat flour	25.12	27.87	29.17
Sucrose	25.12	27.87	29.17
Pasteurised whole eggs liquid	45.73	–	–
Palm oil	4.01	4.45	–
Salt	0.49	0.55	0.57
Chemical raising powder	–	1.10	1.16
Mineral water	–	38.12	39.9

M1, matrix 1 (sponge cake); M2, matrix 2; M3, matrix 3.

Table 2
Composition and physico-chemical characteristics of the flavour compounds of the aromatic formulation

Aroma compounds	Chemical class	g/l in the aroma formulation	MW (g/mol)[a]	BP (°C)[b]	P_v^s (Pa)[c]	Hydrophobicity (log *P*)[d]
Ethyl acetate	Ester	5	88	77.91	12,400	0.86
Ethyl butanoate	Ester	3	116	121.79	1710	1.85
Ethyl hexanoate	Ester	2	144	170.05	2.40[e]	2.83
Methyl cinamate	Ester	2	162	239.90	6.2[e]	2.36
Acetoin	Ketone	6	88	137.57	267[e]	−0.36
Diacetyl	Ketone	3	88	117.70	7570	−1.34
Maltol	Ketone	15	126	267.24	0.00571[e]	−0.19
Dihydrocoumarin	Ketone	5	148	288.73	1.1[e]	0.97
Butyric acid	Acid	5	88	166.84	220	1.07
Hexanoic acid	Acid	1	116	207.76	5.8	2.05
Decanoic acid	Acid	3	97	278.75	0.048	4.02
cis-3-Hexen-1-ol	Alcohol	2	100	165.73	12.5[e]	1.61
Sulfurol	Sulfur alcohol	3	143	259.88	1.228[e]	1.11
Benzaldehyde	Aldehyde	2	106	181.22	16.9	1.71
Vanillin	Aldehyde	200	152	274.30	0.413[e]	1.05
γ-Decalactone	Lactone	8	170	281.72	0.685[e]	2.57
δ-Decalactone	Lactone	10	170	267.24	0.633[e]	2.57
Furaneol	Furane derivative	10	128	258.62	0.125[e]	0.82
α-Citral	Terpene	5	152	170.05	12.2[e]	3.45
Propylene glycol	Alcohol (solvent)	710	76	155.15	17.2	−0.78

[a] Molecular weight.
[b] Boiling point.
[c] Vapour pressure values from EPI Suite™, Copyright 2000, US Environmental Protection Agency.
[d] Expressed by the estimated logarithm of the *n*-octanol/water partition coefficient (from KOWIN v.1.67).
[e] Estimated from MPBPWIN v.1.41.

doughs the aroma mixture was added to obtain a 0.1% (w/w) aromatised dough.

2.3.2. Baking conditions

Each dough (210 g) was placed in an aluminium mould (25× 10 cm) with a Teflon® coating. Baking was achieved in a household electric oven (Rowenta 28L-1500W) for 25 min at 170 °C. Each dough was baked one at a time and always in the same position in the oven. Halfway through cooking (12.5 min), the position of the mould was rotated by 180°.

2.3.3. Storage

After cooking, the matrices were immediately removed from the moulds and were left to cool at ambient temperature. The crust was then removed and the crumb was cut and crushed. Five cakes of the same formulation were crushed together, sampled and stored at −20 °C until analysis.

2.4. Aroma extraction

Solvent assisted flavour evaporation technique (SAFE) was used for the isolation of the aroma compounds from the baked matrices. Twenty grams of frozen matrices were mixed with 50 ml of Milli-Q water and homogenised with an Ultraturrax® for 30 s. The mix was introduced into the SAFE apparatus (Engel et al., 1999) and vacuum distillation (10^{-2} Pa) was performed for two hours. The *head* and *legs* of the apparatus were thermostated at 30 °C. The same temperature was used to heat the distillation vessel by means of a water bath. After vacuum distillation, the water phase containing the volatile compounds was stirred twice with 10 ml of distilled dichloromethane. After liquid–liquid separation, the organic phase was collected and filtered through glass wool and dried over anhydrous sodium sulphate into a graduated flask. Seventy five microlitre of an internal standard; undecane (5 μl/100 ml distilled dichloromethane) was added and was made up using dichloromethane to 25 ml. The extract was concentrated to 1 ml or 500 μl depending on the concentration of the compound in the aroma mixture using a Kuderna-Danish apparatus in a 70 °C water bath.

2.5. Quantification by standard addition method

Quantification was determined by adding known amounts (from 5 to 40 μl) of the aroma mixture before the SAFE extraction to 20 g of crushed matrices samples. Each of them was made up in duplicate.

Simple linear regressions were calculated for each compound and in the three matrices from the following equation:

$$y = a_i x + b_i,$$

where x is the amount of pure compound added (in the added quantity of aroma mixture), y is the corresponding ratio between the peak area of this compound and the peak area of the internal standard (undecane), and i is an index for the type of matrix.

Each regression was calculated using 6–10 values. Three regression models were obtained for each aroma compound, corresponding to M1, ($i = 1$); M2, ($i = 2$) and M3 ($i = 3$). Using the regression models calculated for each compound, the concentration of aroma compounds after baking (x-intercept) was calculated. The percentage of aroma retained was calculated according to this expression

$$\text{Aroma retained (\%)} = (\text{A baked matrix}/\text{A dough}) \times 100,$$

where ‘A dough’ is the amount of aroma compound added to the dough and ‘A baked matrix’ is the amount of aroma compound in the matrices after baking (calculated with the standard addition method).

2.6. Gas chromatography analysis

Sample (1 μl) was injected into a split–splitless injector using an auto-sampler A200S and a GC-8000 chromatograph (Fisons Instruments). The temperature of the injector was 240 °C. An inactivated fused-silica pre-column (30 × 0.32 mm ID, Supelco, Bellefonte, PA, USA) and a fused-silica capillary column (30 m long, 0.25 mm ID, 0.5 μm film thickness) coated with a stationary phase DB-Wax (J&W Scientific, USA) were used. Helium was used as the carrier gas and the chromatographic temperature was programmed from 40 °C for 3 min to 250 °C at a rate of 4 °C/min, with a final isotherm of 15 min. Flame ionisation detector was used at 250 °C.

To confirm the identity of the added compounds in the matrices after baking, a gas-chromatograph–mass spectrometer 5973 network (Agilent Co., Palo Alto, CA, USA) was used with the same column and chromatographic conditions previously described.

2.7. Statistical analysis

Statgraphics v.3 was used to calculate the regression models and the confidence intervals at 95% for the slopes and for the x-intercepts.

3. Results and discussion

3.1. Extraction of aroma compounds by SAFE technique

The SAFE method was chosen as one of the softest methods in preserving the added aroma compounds and because other authors have shown that it could be a useful method to extract aroma compounds in oily matrices (Engel et al., 1999). In preliminary tests performed in our laboratory with this method, we obtained better results than with simultaneous extraction of steam distillates by dichloromethane (SDE method). Whilst the sample was being heated during the SDE method, there was the production of a great number of other volatile compounds that masked the added aroma compounds (data not shown).

Fig. 1 shows the chromatogram obtained from a SAFE extract of the flavoured matrix M1 (sponge cake). This method allowed the extraction of the majority of the added aroma compounds; nevertheless, some of them have not been quantified because of their co-elution with the solvent, such as ethyl acetate. Other compounds such as butyric acid and decanoic acid had very small peak areas in the

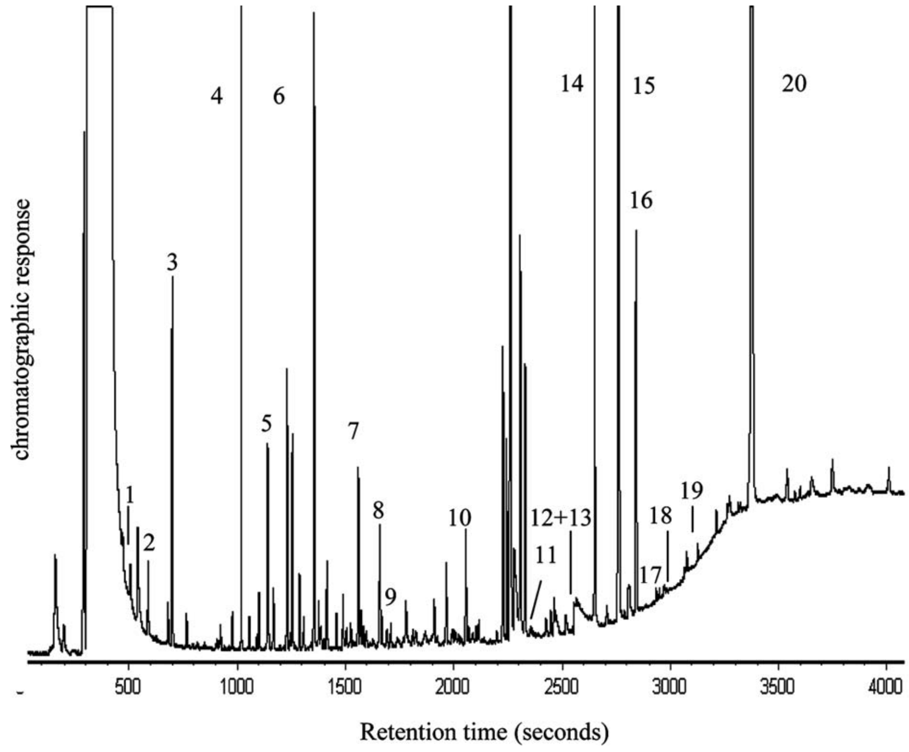

Fig. 1. GC-FID chromatogram of a SAFE extract from the flavoured sponge cake (matrix M1). The numbers correspond to the added aroma compounds: (1) diacetyl; (2) ethyl butanoate; (3) internal standard; (4) ethyl hexanoate; (5) acetoin; (6) *cis*-3-hexen-1-ol; (7) benzaldehyde; (8) propylene glycol; (9) butanoic acid; (10) α-citral; (11) hexanoic acid; (12) maltol; (13) furaneol; (14) methyl cinnamate; (15) γ-decalactone; (16) δ-decalactone; (17) dihydrocoumarin; (18) decanoic acid; (19) sulfurol; (20) vanillin.

chromatograms. This could be because they were added to the dough in very small amounts that are commonly used in commercial products, but they could have also been poorly extracted from the matrices since they may interact strongly with amylose (Le Bail, Biais, Pozo-Bayón, & Cayot, 2004). Hexanoic acid has not been quantified because it had the same retention index as another compound produced during baking.

Other compounds of the aroma formula such as furaneol, maltol, dihydrocoumarin and sulfurol have been identified in the matrices using mass spectrometry, although their quantification has not been possible because of their small peak areas. Furaneol is a very polar and labile flavour compound that could be lost during baking. Engel et al. (1999) have also shown that using the SAFE technique the yield of extraction of this compound decreased from 100% in a solvent solution to 4.3% when oil was added to the same solution at 50%. Maltol, dihydrocoumarin and sulfurol, which were added to the dough in very low concentrations, might have been hardly extracted with dichloromethane after the vacuum step.

Finally from the 20 compounds added to the dough (19 aroma compounds and propylene glycol), 13 of them were quantified. It has been shown that some aroma compounds such as acetoin, γ-decalactone α-citral and vanillin that are not greatly extracted using other techniques such as SDE or direct extraction by solvents (Prost et al., 1993) can be extracted using the SAFE technique.

3.2. Performance of the standard addition method in the matrices of different composition

In order to understand the effects of the matrix composition on the performance of the standard addition method, the quantification was carried out in the sponge cake (M1) and in the two other simplified matrices M2 and M3. A linear regression model for each compound in the three matrices was then calculated.

Results of the linear regressions for the three matrices are shown in Table 3. The determination coefficients (r^2) were considered good (above 0.8) in 28 of 36 regressions obtained and indicated that the fits were acceptable and lead to a good precision in the quantification. The values of the determination coefficients for propylene glycol and vanillin were not acceptable and these compounds have not been quantified. The small correlation found for propylene glycol could be explained by its poor recovery from the dichloromethane used for the liquid–liquid extraction after the vacuum procedure. In spite of vanillin having the highest peak areas in the chromatograms, the lack of fit in the three regression models for this compound could be explained by its ability to be adsorbed by food polymers (Hansen & Heinis, 1991; Ng, Hoehn, & Bushuk, 1989) that could occur during the addition of the standard solution before the extraction.

The 95% confidence interval for the slopes (CI) for all the compounds in the three matrices is also shown in Table 3. When the estimated slope for one compound in one matrix is included in the confidence interval for the theoretical slopes in the other two matrices, there are not any significant differences between the slopes. In this way, the theoretical slope calculated for M1 (a_1) was compared with the slopes calculated for M2 (a_1/a_2) and M3 (a_1/a_3). The comparison between the slopes of the two simple matrices (a_2/a_3) was also carried out. Non-significant differences were found between the slopes obtained for γ-decalactone in the three matrices whereas the slopes were significantly different for benzaldehyde and methyl-cinnamate. Table 4 summarises the differences between the slopes for each compound in the three matrices. For the majority of the aroma compounds the slopes of the regressions were similar in M1 and M2 and also higher than in M3. Only the slopes calculated for acetoin, methyl-cinnamate and δ-decalactone were higher in M1, while the slope of benzaldehyde was significantly higher in M2 than in M1.

The differences between the slopes in the three regression models are related with the interactions between the ingredients and the aroma compounds added after baking (during the standard addition) and also with the differences in aroma loss during processing. As the greatest differences are between M1 and M2 compared with M3, it seems that the influence produced by the palm oil is higher than the influence produced by the eggs. These differences need to be considered in the quantification of the aroma compounds in the matrices.

3.3. Quantification of aroma compounds in the matrices after processing

Each linear regression model was applied to quantify the aroma compounds in the three matrices after processing. Table 5 shows the concentration values estimated for each compound (x-intercept). In the sponge cake (M1) the concentration calculated for ethyl hexanoate, acetoin, *cis*-3-hexen-1-ol, benzaldehyde, and γ-decalactone ranged between 0.57 mg/l for *cis*-3-hexen-1-ol and 13.55 mg/l for γ-decalactone. For diacetyl, ethyl butanoate, α-citral, methyl-cinnamate and δ-decalactone the estimated concentration was not statistically different from zero (from 95% prediction limits for x at $y = 0$ obtained with Statgraphics program). In matrix M2, acetoin, *cis*-3-hexen-1-ol and γ-decalactone have shown concentration values similar to matrix M1 but methyl cinnamate was higher in M2 (1.61 mg/l). Nevertheless, all the concentration values estimated for the aroma compounds in matrix M3 were not statistically different from zero. This shows the weak concentration of aroma compounds that remain in this matrix after processing. For better quantification, a new calibration experiment could be realised with a greater number of measurement points to narrow down the width of the prediction limits for x (Imhof & Bosset, 1994).

From the concentration values estimated for the aroma compounds in the matrices after processing, the percentage of aroma retained compared with the amount of aroma

Table 3
Linear regression between the amount of pure compound added to the matrices (mg/kg) and the chromatographic response (y = peak area of compound/peak area of internal standard) obtained with the standard addition method

Aroma compounds	Matrix	Added standard (min–max) (mg/kg)	Linear regression $y = ax + b$		r^2 [a]	CI [b]
Diacetyl	M1	(0.73–3)	$a_1 = 9.85$	$b_1 = 0.039$	0.930	(7.13–12.46)
	M2	(0.75–3)	$a_2 = 10.83$	$b_2 = -0.004$	0.957	(8.54–13.11)
	M3	(0.75–3)	$a_3 = 6.01$	$b_3 = -0.028$	0.864	(3.83–8.19)
Ethyl butanoate	M1	(0.75–3)	$a_1 = 87.43$	$b_1 = 0.081$	0.981	(77.52–99.35)
	M2	(0.75–3)	$a_2 = 82.50$	$b_2 = -0.344$	0.936	(61.00–103.99)
	M3	(0.75–3)	$a_3 = 49.77$	$b_3 = -0.104$	0.899	(33.16–66.37)
Ethyl hexanoate	M1	(1.00–2.0)	$a_1 = 79.0$	$b_1 = 1.76$	0.918	(46.24–111.76)
	M2	(0.5–2.0)	$a_2 = 71.31$	$b_2 = 0.19$	0.923	(53.02–89.60)
	M3	(0.5–2.0)	$a_3 = 51.14$	$b_3 = -0.035$	0.906	(34.72–67.56)
Acetoin	M1	(6.0–12)	$a_1 = 7$	$b_1 = 0.441$	0.950	(4.77–9.22)
	M2	(1.5–6.0)	$a_2 = 4.41$	$b_2 = 0.388$	0.836	(2.46–6.36)
	M3	(1.5–6.0)	$a_3 = 3.83$	$b_3 = 0.106$	0.819	(2.03–5.63)
cis-3-Hexen-1-ol	M1	(0.5–2.0)	$a_1 = 117.42$	$b_1 = 1.33$	0.970	(97.09–137.76)
	M2	(0.5–2.0)	$a_2 = 125.18$	$b_2 = 0.821$	0.923	(92.98–157.37)
	M3	(0.5–2.0)	$a_3 = 94.87$	$b_3 = 0.166$	0.961	(75.92–113.81)
Benzaldehyde	M1	(2.0–4.0)	$a_1 = 12.312$	$b_1 = 0.70$	0.883	(6.10–18.51)
	M2	(0.5–2.0)	$a_2 = 38.74$	$b_2 = 0.130$	0.941	(25.37–52.11)
	M3	(0.5–2.0)	$a_3 = 21.54$	$b_3 = 0.068$	0.861	(12.92–30.16)
α-Citral	M1	(1.5–10)	$a_1 = 19.55$	$b_1 = 0.426$	0.765	(10.72–28.39)
	M2	(1.25–5)	$a_2 = 32.09$	$b_2 = 0.482$	0.877	(18.28–45.90)
	M3	(1.5–6)	$a_3 = 17.43$	$b_3 = 0.142$	0.887	(11.24–23.63)
Methyl cinnamate	M1	(2.0–4.0)	$a_1 = 131.75$	$b_1 = 2.181$	0.845	(53.67–209.82)
	M2	(0.5–20)	$a_2 = 55.34$	$b_2 = 1.783$	0.898	(36.79–73.903)
	M3	(0.5–2.0)	$a_3 = 85.72$	$b_3 = 0.101$	0.903	(57.73–113.71)
γ-Decalactone	M1	(8.0–16)	$a_1 = 24.78$	$b_1 = 6.716$	0.869	(11.01–38.54)
	M2	(4.0–8.0)	$a_2 = 30.21$	$b_2 = 2.789$	0.950	(23.29–37.14)
	M3	(4.0–8.0)	$a_3 = 28.37$	$b_3 = 1.135$	0.812	(9.475–47.27)
δ-Decalactone	M1	(10–20)	$a_1 = 7.31$	$b_1 = 1.426$	0.743	(−0.57–15.2)
	M2	(2.5–10)	$a_2 = 6.58$	$b_2 = 0.113$	0.930	(4.78–8.37)
	M3	(5–20)	$a_3 = 4.45$	$b_3 = 0.003$	0.944	(2.95–5.94)
Vanillin	M1	(50–200)	$a_1 = 2.13$	$b_1 = 14.941$	0.458	(−0.08–5.34)
	M2	(50–100)	$a_2 = 5.24$	$b_2 = 3.337$	0.692	(1.91–8.57)
	M3	(50–200)	$a_3 = 4.68$	$b_3 = -1.640$	0.751	(1.99–7.36)
Propylene glycol (solvent)	M1	(177–710)	$a_1 = 0.007$	$b_1 = 0.166$	0.407	(0.090–0.24)
	M2	(177–710)	$a_2 = 0.001$	$b_2 = 0.074$	0.080	(−0.004–0.007)
	M3	(177–355)	$a_3 = 0.005$	$b_3 = 0.013$	0.510	(−0.002–0.013)

[a] Determination coefficient.
[b] 95% Confidence interval for the slopes.

compounds added to the dough was calculated (Table 5). In the sponge cake (M1) the percentage of retention was between 28.64% for *cis*-3-hexen-1-ol and 169.59% for γ-decalactone. De Roos and Graf (1995) have also determined for δ-2 decalactone a higher value of retention (80%) in cakes baked for 40 min. Moreover, the highest values of retention that we have obtained for the two lactones could be explained by their generation during baking as has been reported (Maga, 1976). Nevertheless, De Roos and Graf (1995) showed higher retention for *cis*-3-hexen-1-ol (50%). After processing, M1 retained 55.78% % of the ethyl hexanoate added to the dough, which is higher to that determined by Heiderich and Reineccius (2001) in flavoured cookies heated at 191 °C during 10 min. For ethyl butanoate the same authors have indicated losses of up to 70% produced mainly during the preparation of the dough but also during baking. For this compound, we have determined more important losses (minor retention) that could be explained by the lower amount of aroma that has been used to aromatise the dough (6 mg/kg) compared with the amount used in their study (600 mg/l). In spite of its high vapour pressure, benzaldehyde was one of the most retained compounds in the sponge cake (143.72%). In previous experiments carried out in our laboratory benzaldehyde has been identified in the same matrix (M1) without flavouring. It has been reported that this compound is one of the quantitatively most important volatile compounds of fresh and cooked eggs (Umano, Hagi, Shoji, & Shibamoto, 1990; Warren, 1994; Warren, Larick, & Ball, 1995) and moreover, it can be formed from the Strecker

Table 4
Aroma compounds with significant differences (at 5% level) between the slopes from the regressions obtained with the application of the standard addition method to the matrices M1, M2 and M3

Aroma compounds	$a_1 > a_2$	$a_1 > a_3$	$a_2 > a_1$	$a_2 > a_3$	$a3 > a1$	$a3 > a_2$
Diacetyl		*		*		
Ethyl butanoate		*		*		
Ethyl hexanoate		*		*		
Acetoin	*	*				
cis-3-Hexen-1-ol		*		*		
Benzaldehyde			*	*	*	
α-Citral				*		
Methyl cinnamate	*	*				*
γ-Decalactone				*		
δ-Decalactone	*	*				

a_1, a_2 and a_3: slopes from the regressions obtained with the application of the standard addition method in the matrices M1, M2 and M3.
The table shows when the slope of one compound is higher in one matrix than in the other.

Table 5
Concentration of aroma compounds in the three matrices after processing (elaboration and baking) and percentage of aroma retained compared with the quantity of aroma added to the dough

Aroma compounds	mg/kg added to the dough			mg/kg in the matrices after processing[a]			% Aroma retained after processing[b]		
	M1-dough	M2-dough	M3-dough	M1-matrix	M2-matrix	M3-matrix	M1-matrix	M2-matrix	M3-matrix
Diacetyl	2.99	3.00	3.06	0.20[c]	0.02[c]	0.16[c]	6.69[c]	0.67[c]	5.23[c]
Ethyl butanoate	2.99	3.00	3.06	0.05[c]	0.19[c]	0.10[c]	1.67[c]	6.33[c]	3.27[c]
Ethyl hexanoate	1.99	2.00	2.04	1.11	0.13[c]	0.03[c]	55.78	6.50[c]	1.47[c]
Acetoin	5.99	6.00	6.12	3.15	4.40	1.38[c]	52.59	73.33	22.55[c]
cis-3-Hexen-1-ol	1.99	2.00	2.04	0.57	0.32	0.09[c]	28.64	16.00	4.41[c]
Benzaldehyde	1.99	2.00	2.04	2.86	0.17[c]	0.16[c]	143.72	8.50[c]	7.84[c]
α-citral	4.99	5.00	5.10	1.09[c]	0.75[c]	0.41[c]	21.84[c]	15.00[c]	8.04[c]
Methyl cinnamate	1.99	2.00	2.04	0.83[c]	1.61	0.06[c]	41.71[c]	80.50	2.94[c]
γ-decalactone	7.99	8.00	8.16	13.55[d]	4.62[d]	2.00[c]	169.59[d]	57.75[d]	24.51[c]
δ-decalactone	9.99	10.00	10.20	9.75[c]	0.86[c]	0.34[c]	97.60[c]	8.60[c]	3.33[c]

[a] Calculated as the x-intercept of the regression line from the standard addition method.
[b] Calculated from the concentration estimated in the matrices after processing compared with the quantity of aroma added to the dough.
[c] The estimated concentration is not statistically different from zero at 95% confidence level.
[d] Significant differences between the values (calculated from the x prediction limits at 95% confidence level).

degradation of the amino acid phenylglycine during heating (Whitfield, 1992). It would then have already been present in the dough before its aromatisation or be formed during baking and its concentration would therefore be overestimated.

In matrix M2 the retention of acetoin (73.33%) and *cis*-3-hexen-1-ol (16.00%) was not significantly different than in the sponge cake (M1). Nevertheless γ-decalactone was less retained (57.75%). One possible reason that has been indicated before could be the generation of lactones during baking, mainly in the matrices with more fatty ingredients (eggs in M1), but also as it has been shown in different works (Brauss et al., 1999; De Roos & Graf, 1995; De Roos & Mansencal, 2003; De Roos, 2003; Heiderich & Reineccius, 2001; Reineccius & Whorton, 1990) the flavour loss during baking is related to the partition coefficient and the resistance to mass transfer of the compound in the matrix. Therefore, γ-decalactone that is the most hydrophobic compound of the aroma mixture will be more retained by the matrix with the higher fat content (M1) and less evaporated during baking. On the contrary, methyl cinnamate was more retained in M2 than in M1. De Roos and Mansencal (2003) have shown that in carbohydrate matrices the resistance to mass transport for some hydrophobic aroma compounds would be higher in matrices with less fatty material because the aroma compounds could be immobilised in the carbohydrate matrix and this effect would be higher than aroma loss with water evaporation.

As it was expected, the absence of fatty material in matrix M3 dramatically influenced the retention of the aroma compounds. The percentage of each aroma compound that remained in this matrix after processing was not statistically different from zero.

4. Conclusion

In this study, it was shown that solvent assisted flavour evaporation technique allows the extraction of all of the aroma compounds of a baked cereal matrix containing a complex aromatic formulation without producing any transformations of the added aroma compounds. The standard addition method allows the quantification of the added compounds considering the differences in the composition of the matrices, which influences the degree of interactions of the aroma compounds in the matrix and the loss of the aroma compounds during baking. Due to the complexity of the aroma mixture used in this study, mainly because of the wide range of chemical classes and the small quantity employed to aromatise the dough (usually used in industrial flavoured cakes), it has not been possible to quantify all the added aroma compounds. Nevertheless in cereal matrices aromatised with a more simple aromatic mixture, the combination of these two methods would be a useful tool for the quantification of the aroma compounds retained in the matrices after processing.

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