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Interactions between non-volatile water-soluble molecules and aroma compounds in Camembert cheese

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Abstract

Interactions between selected aroma compounds and non-volatile water-soluble molecules were studied using dynamic headspace-gas chromatography. A model water-soluble extract (MWSE), previously constructed in gustatory and physico-chemical accordance with the crude Camembert cheese WSE, allowed the contribution of non-volatiles to the headspace composition of volatile compounds to be assessed. The presence of the MWSE increased the headspace concentration of 2-heptanone, 1-octen-3-ol and 3-methylbutanol, showing that these three volatile compounds were released by MWSE. Omission tests performed on MWSE allowed for the impact of each MWSE component on aroma compounds release to be determined. The releasing influence of minerals appeared as the main effect observed for the three volatiles, despite some retention phenomena due to other MWSE components also occurring. In the case of 2-undecanone, 2-nonanol, 2,4-dithiapentane and ethylhexanoate, which were not affected by the presence of MWSE, some significant compensatory effects were observed. Whereas amino acids had no significant effect, minerals might cause their release and the presence of peptides can either decrease or increase headspace concentration of aroma compounds. Possible antagonistic effects between MWSE components are discussed.

Keywords: Interaction; Omission tests; Camembert cheese; Water-soluble extract; Dynamic headspace; Aroma compounds

1. Introduction

The acceptability of food by the consumer mainly depends on its sensory qualities. An important factor in this context is the flavour. The numerous compounds involved in cheese flavour are mainly derived from three major metabolic pathways:lactose, lipid and protein catabolism. The resulting molecules are principally alcohols, ketones, esters, fatty acids, sulfur compounds and lactones (Kubickova & Grosh, 1998a, 1998b; Dumont, Roger, Cerf, & Adda, 1974; McSweeney & Sousa, 2000; Molimard & Spinnler, 1996).

Apart from parameters linked to subject physiology and psychology, aroma perception depends mainly on the nature and concentration of the volatiles but also on their availability, which can be modulated by physico-chemical interactions between food components. A number of different interactions have been proposed to explain the association of volatiles with non-volatile compounds (Bakker, 1995). Widder (1996) showed, by a Headspace-GC-Olfactometry and Headspace-GC-FID analysis, that the headspace concentration of aroma compounds changed in direct correlation with the fat content of a model emulsion. In the same way, Piraprez, Herent, and Collin (1998) showed that the retention of methylketones, esters, aldehydes and sulphur compounds, in fresh cheese increased with the triolein content. A range of volatiles such as alcohols, aldehydes, ketones can interact with proteins. Their binding is dependent on the macromolecule conformation. Thus, factors modifying this conformation are able to affect the interaction (Langourieux & Crouzet, 1997; Pelletier, Sostmann, & Guichard, 1998). In contrast, few researchers have studied the influence of small water-soluble molecules on cheese aromatic compounds.

Some studies done on model mixtures have demonstrated the modulating effect of non-volatile compounds on headspace concentration of aromatic molecules. Salting-out is known to influence the partial pressure of

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volatile constituents. Jennings (1965) showed, for instance, that radioactivity, due to the quantity of C^{14}-labelled ethyl acetate increased in headspace in accordance with NaCl concentration. Schinneller, Dougherty, and Biggs (1972) showed an increase of octanal release due to the presence of 5′-nucleotides. Some authors (Delahunty & Piggot, 1995; Solms, 1986) have suggested that, in cheese, interactions between small water-soluble molecules of the water-soluble extract (WSE) and aroma compounds may occur.

The relative contributions of an individual components in a mixture context may be shown using omission tests (Fujimura, Kawano, Koga, Takeda, Kadowaki, & Ishibashi, 1995). Starting with a synthetic model mixture, these tests consist in evaluating the sensory effect of omitting one or several components of the complete solution. Developed for the study of goat cheese and Camembert cheese, this method allowed for the relative impact of WSE components on their taste to be highlighted (Engel, Nicklaus, Garem, Septier, Salles, & Le Quéré 2000; Engel, Nicklaus, Septier, Salles, & Quéré, 2000; Engel, Septier, Feyen, Nicklaus, Salles, & Quéré, 2000). These previous studies enabled the authors to point out taste interactions between compounds, such as additive, synergistic and masking effects, using a model WSE (MWSE) which had been constructed in gustatory and physico-chemical accordance with the crude WSE. Using omission tests on this model mixture, it will be interesting to test the method from a physico-chemical point of view, to identify possible interactions between non-volatile water-soluble molecules (included in the WSE) and Camembert cheese aroma compounds.

The first objective of the study was to investigate the impact of the WSE on selected key aromatic compounds of Camembert cheese. In a second step, the use of omission tests on a previously elaborated model WSE (MWSE) of Camembert cheese (Engel, Septier et al., 2000), should allow the relative impact of each WSE component on the release of aroma compounds to be evaluated.

2. Materials and methods

2.1. Chemicals

2.1.1. Water

Pure water was obtained from a milliQ system® (Millipore, Bedford, MA).

2.1.2. Non-volatile compounds

The following food grade chemical substances were purchased from commercial suppliers: L-alanine, L-arginine, L-asparagine monohydrate, L-aspartic acid, L-citrulline, L-cysteine, L-γ-amino-butyric acid, L-glutamic acid, L-glutamine, L-glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, L-methionine, L-ornithine monochlorhydrate, L-phenylalanine, L-proline, L-serine, L-threonine, L-tryptophan, L-tyrosine and L-valine (Rexim, Courbevoie, France), D-lactose monohydrate, lactic acid, sodium chloride, potassium chloride, calcium chloride dihydrate, magnesium chloride hexahydrate, disodium hydrogen phosphate-dodecahydrate, monosodium dihydrogen phosphate dihydrate and sodium hydroxide (Merck, Darmstadt, Germany), potassium aluminium sulphate (Sigma, St Quentin Fallavier, France) L-monosodium glutamate and capsaicin (Fluka, St Quentin Fallavier, France).

2.1.3. Aromatic compounds

The aroma compounds were purchased from commercial suppliers: Aldrich, IFF, Fluka. The chromatographic purity was in all cases greater than 95%. The compounds were selected among the major Camembert cheese components according to their strong odour activity values, obtained by Molimard and Spinnler (1996). They were divided into two groups according to their volatility and the way to analyse them. Table 1 summarises the composition of the two aroma solutions.

2.2. Extraction and purification of WSE peptides

The detailed procedures are described in Engel, Septier et al. (2000).

2.2.1. Extraction procedure

The frozen cheese portions (22.5 kg) were grated, dispersed in pure water (w/w : 1/2) and homogenised for 4 min in a 1094 homogeniser (Tecator, Höganäs, Sweden). The suspension was centrifuged at 20,000 ***g*** at 4 °C for 30 min. Three phases were separated:a fatty upper-layer, a liquid fraction including water-soluble molecules called C20000 and a pellet of proteins. After filtration on gauze, the C20000 was recovered. All these fractions were stored at −80 °C until further use.

2.2.2. Microfiltration procedure

All the C20000 were pooled and submitted to tangential microfiltration in a pilot apparatus equipped with two 0.05 μm membrane modules of 0.9 m^2 each (wide volume = 15 l ; INRA, Laboratoire de Recherches de Technologie Laitière, Rennes, France). The retentate was rinsed with 4×11 l of osmosed water by successive diafiltrations. This process allowed for the recovery of 19.7 kg of microfiltration retentate, named RUF0.05, and 63.2 kg of microfiltration permeate, considered to be the WSE. All the obtained fractions were frozen at −80 °C until further use.

2.2.3. Ultra- and nanofiltration

Fifty-six kilograms of the diluted WSE were pooled and successively submitted to two tangential ultrafiltrations

(respectively, 10 and 1 kDa cut-off) with polysulfone membrane (Millipore, Bedford, MA) and tangential nanofiltration (0.5 kDa cut-off) using a Nanomax 50 membrane (Millipore, Bedford, MA) according to Engel, Nicklaus, Garem et al. (2000). Ultra- and nanofiltration were performed in a pilot apparatus (MSP 006239 Prolab, Millipore, Bedford, MA), as described by Garem et al. (1996). The filtration temperature and the transmembrane pressure were, respectively, maintained at approximately 15 °C and 4 Pa. At each step, an aliquot of retentate and permeate was kept for physico-chemical measurements. The retentates of 10, 1 and 0.5 kDa nanofiltration called, respectively RUF10, RUF1 and RNF were rinsed with 7.9, 5.6 and 5.7 kg osmosed water recovered at each step in the corresponding permeate. The retentates RUF10, RUF 1 and RNF were freeze-dried for further construction of the MWSE or incomplete MWSEs.

2.3. Construction of the MWSE and incomplete MWSEs

2.3.1. MWSE

The MWSE was constructed in physicochemical and gustatory accordance with the cheese WSE, using synthetic chemicals and ultra- and nanofiltration retentates as sources of peptides. The MWSE-P was the synthetic model mixture obtained by omitting all peptides from the MWSE. Due to the remaining amount of compounds, other than peptides present in the retentates, it was necessary to re-adjust the other constituent concentration in the case of peptides omission. The compositions of both MWSE and MWSE-P are given in Table 2.

Table 1
Composition of the aroma solutions

Aroma compounds	Concentrations in Camembert cheese[a] (mg/kg)	Concentrations in aroma solutions (mg/kg)
Solution 1		
Butyric acid	98.0	104
Hexanoic acid	57.5	57.9
Octanoic acid	32.0	35.0
δ-decalactone	8.36	17.6
γ-dodecalactone	7.62	18.5
2-phenylethanol	1.18	2.67
Solution 2		
2-pentanol	0.500	0.700
2-nonanol	0.630	0.650
1-octen-3-ol	0.130	0.155
2-phenylethanol	1.18	22.5
3-methylbutanol	1.26	1.20
2-heptanone	1.26	1.33
2-nonanone	2.40	2.20
2-undecanone	0.530	0.425
Ethyle butanoate	–[b]	0.300
Isoamyle acetate	–[b]	0.210
Ethyle hexanoate	–[b]	0.115
Dimethyldisulfide	–[b]	0.100
2.4-dithiapentane	–[b]	0.100

[a] Molimard (1994)
[b] Unknown.

2.3.2. Incomplete MWSEs

The incomplete model mixtures were prepared without adding one or several compounds present in the MWSE. The constructed MWSEs were: MWSE-P (-Peptides), MWSE-AA (-Amino acids), MWSE-S (-Mineral Salts).

2.4. Dynamic headspace analysis (all measurements were done in triplicate)

2.4.1. Sample preparation

First, aroma solutions and MWSE or incomplete MWSE were prepared at twice the working concentration.

Table 2
Composition of MWSE and MWSE-P[a]

	MWSE (g/kg MWSE)	MWSE-P (g/kg MWSE-P)
Amino acids		
ASP	0.08	0.22
THR	0.12	0.13
SER	0.16	0.18
ASN	0.14	0.16
GLU	0.23	0.62
GLN	0.46	0.57
PRO	0.44	0.50
GLY	0.08	0.08
ALA	0.12	0.13
CIT	0.15	0.24
VAL	0.27	0.37
MET	0.11	0.13
ILE	0.17	0.26
LEU	0.50	0.70
TYR	0.27	0.33
PHE	0.35	0.42
HIS	0.44	0.66
ORN	0.04	0.06
LYS	0.50	0.64
ARG	0.04	0.05
Total	4.67	6.45
Galactose	0.12	0.17
Na_2HPO_4	0.37	0.70
Mineral salts		
NaCl	13.3	13.3
KCl	1.12	1.22
$CaCl_2$	0.69	0.89
$MgCl_2$	0.09	0.13
Lactic acid	2.72	3.51
RUF10	20.4	
RUF1	28.24	
RNF	35.2	
NaOH	0.68	0.87

[a] MWSE, model water-soluble extract; MWSE-P, MWSE-peptides; RUF10, RUF1, RNF: Retentates obtained, respectively, by 10 kDa cutoff and 1 kDa cutoff ultrafiltration then 0.5 kDa nanofiltration of the water-soluble extract of Camembert cheese.

After a 20-min homogenisation, 10 ml of aroma solution (1 or 2) and 10 ml of MWSE or incomplete MWSE were put into a 500-ml flask. The mixture was then put into a water-bath at 25 °C for 30 min in order to reach the liquid phase/gaseous phase equilibrium.

2.4.2. Sample collection

Volatile compounds in the headspace were purged at 25 °C with nitrogen at 40 ml/min for 15 min (mixture made with the aroma solution 1) or 7 min 30 (aroma solution 2) and trapped on a 140 mg Tenax TA trap (160×3 mm i.d., 20–35 mesh). Water was eliminated with a backflush of nitrogen at 40 ml/min for 1 min (aroma solution 2) or 2 min (aroma solution 1). The desorption of the volatiles was performed with a CP-4010 PTI/TCT thermal desorption cold trap injector (Chrompack, Middleburg, The Netherlands). The Tenax trap was placed inside the desorption oven. Volatile compounds were then desorbed from the Tenax trap with N_2 (40 ml/min) at 240 °C for 20 min. The desorbed volatiles were cryofocused on a fused silica trap cooled to −130 °C with liquid nitrogen. The cryotrap was raised to 250 °C for 1 min and volatiles were injected into the chromatograph. The trap was then backflushed at 260 °C for 20 min.

2.4.3. GC analysis

The compounds of the aroma solutions, put in total or incomplete MWSE, were analysed with a Hewlett-Packard Model 5890 Series II gas chromatograph equipped with a DBWAX column (30×0.32 mm i.d., 0.5 μm film thickness, JW Scientific Inc., Folsom, USA). Hydrogen was used as carrier gas at a velocity of 37 cm/s at 143 °C. The FID detector was maintained at 250 °C and the oven temperature maintained at 40 °C for 10 min and then increased at 5 °C/min to a final temperature of 220 °C, maintained for 10 min. Data were recorded and analysed with a laboratory-made hardware and software data system called Cocowin® (Almanza R., UMRA-INRA, Dijon, France).

2.4.4. GC–MS analysis

The compounds of the aroma solution 1, mixed with MWSE or incomplete MWSE, were analysed using the previously described chromatographic apparatus coupled to a 5970 Mass Selective Detector (Hewlett Packard), in order to improve the sensitivity and specificity of the method. The quadripole mass selective detector, with an electron impact ionisation of 70 eV operated in the SIM (Selected Ion Monitoring) mode. The carrier gas was helium and its velocity was 35 cm/s at 143 °C. Oven temperature was programmed from 50 to 240 °C at a rate of 5 °C/min and held at 240 °C for 5 min. Data were recorded and analysed with the HP-Chemstation Unix Software.

2.5. Evaluation of the interactions

2.5.1. Interactions between aroma compounds and the whole MWSE

Two samples, consisting of 10 ml aroma solution mixed with 10 ml of water (reference), and 10 ml of the same aroma solution mixed with 10 ml of the MWSE, were compared for their headspace composition. The MWSE effect is directly observed by comparing the chromatographic peak areas of the two samples. For each aroma compound, a significant difference between the two solutions is expressed by the percentage of the difference between the peak areas of the reference and of the sample in comparison with the peak area of the reference.

2.5.2. Relative contribution of each MWSE component: omission tests

The same procedure was used. The reference solution was MWSE and the sample was an incomplete MWSE where one or several components were omitted. For each omitted MWSE component, the sample was compared to the reference. The impact of each omitted compound was also evaluated in the same way as described above

2.6. Statistics

The comparisons of headspace concentration between the mixtures were analysed by paired Student's *t*-test using a Statbox Pro statistical package version 2.5a (Grimmer Logiciels, Paris, France).

3. Results and discussion

3.1. Impact of the WSE on the volatile fraction

3.1.1. Analysis of aroma solution 1

No significant difference was observed between the chromatographic peak areas of the pure water and MWSE for the aroma solution 1.

3.1.2. Analysis of aroma solution 2

In the presence of the MWSE, the over-release in the headspace reaches 20% for 3-methylbutanol whereas it was weaker for 1-octen-3-ol (14%) and 2-heptanone (12%) (Table 3). Therefore, the MWSE appeared to significantly favour the release of these three compounds.

In order to determine which WSE component was involed in these release phenomena, omission tests were performed on the MWSE. The results are presented in Fig. 1 for 2-heptanone. Neither amino acid nor peptide omissions caused any significant modification in the headspace. In the case of mineral omission, a significant decrease (28%, $P < 0.06$) indicates a release phenomenon

due to their presence. This effect, probably due to salting-out has already been mentioned by numerous authors (Dubois, Lubbers, & Voilley, 1995; Jennings, 1965; Voilley, Simatos, Loncin, 1977) and may explain the global release observed with the MWSE, though the concentration of salts was weak in comparison with other studies where this effect was pointed out (Nelson & Hoff, 1968; Voilley et al., 1977).

Some differences were observed in 3-methylbutanol headspace concentration between the MWSE and the incomplete MWSEs (Fig. 2). The omissions of peptides and mineral salts lead, respectively, to a significant increase ($P<0.1$) and to a significant decrease ($P<0.06$) of 3-methylbutanol headspace concentration. As for 2-heptanone, mineral salts should be involved in a release effect because their omission caused a 41% decrease in 3-methylbutanol headspace concentration. The release caused by the presence of salts may also explain the global release observed with MWSE. In contrast to the case of 2-heptanone, peptides may be responsible for a retention effect on 3-methylbutanol because their omission increased (to 28%) its headspace concentration (Fig. 2).

Table 3
MWSE release effect on three volatiles

Aroma compounds	Observed effect with MWSE[a]
1-octen-3-ol	Release of 14%*
3-methylbutanol	Release of 20%*
2-heptanone	Release of 12%*

[a] Release (%), percentage of the difference between the peak areas of the reference and of the sample for each volatile component.
* $P<0.05$

The individual omission of RUF10, RUF1 and RNF indeed allowed this hypothesis to be confirmed. Whereas RUF10 and RNF had no effect, RUF1 omission significantly ($P<0.05$) increased the 3-methylbutanol headspace concentration. These results indicate that some peptides of molecular weight ranging from 1 to 10 kDa may effectively be involved in a retention effect. However, even if we admit the existence of such a retention, it appears to be negligible in comparison with the global release effect, essentially due to salts.

Fig. 3 shows the omission results obtained for 1-octen-3-ol. A very significant decrease ($P<0.01$) of the

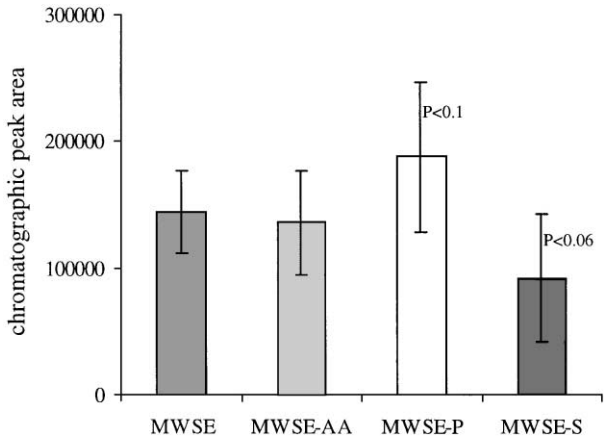

Fig. 2. Modification of the concentration in the headspace of 3-methylbutanol due to the omission tests performed on the MWSE: comparison of the chromatographic peak areas between the MWSE and each incomplete MWSE. The results of the corresponding *t*-tests are given at the top of each bar when it is significant. MWSE, model water-soluble extract; AA, amino acids; P, peptides; S, mineral salts.

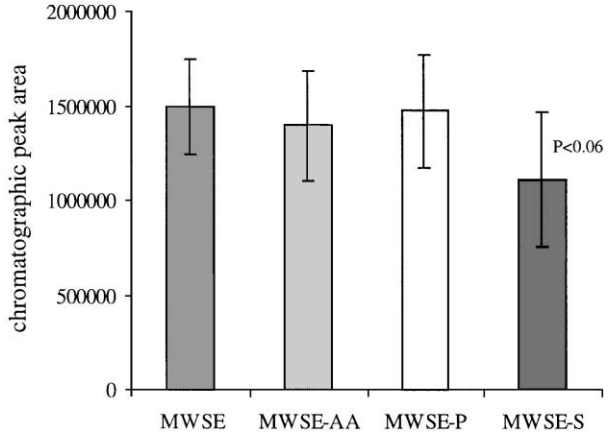

Fig.1. Modification of the concentration in the headspace of 2-heptanone due to the omission tests performed on the MWSE: comparison of the chromatographic peak areas between the MWSE and each incomplete MWSE. The results of the corresponding *t*-tests are given at the top of each bar when significant. MWSE, model water-soluble extract; AA, amino acids; P, peptides; S, mineral salts.

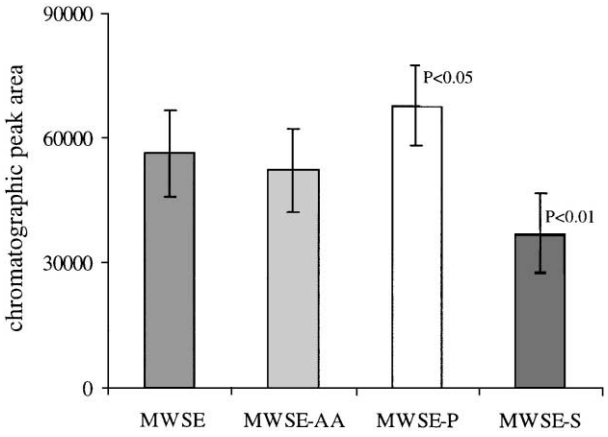

Fig. 3. Modification of the concentration in the headspace of 1-octen-3-ol due to the omission tests performed on the MWSE: comparison of the chromatographic peak areas between the MWSE and each incomplete MWSE. The results of the corresponding *t*-tests are given at the top of each bar when significant. MWSE, model water-soluble extract; AA, amino acids; P, peptides; S, mineral salts.

headspace concentration of this compound (40%) was observed when minerals were omitted. That indicated that they contributed to the whole MWSE effect. The omission of peptides showed a significant increase ($P<0.05$) of 1-octen-3-ol headspace concentration, suggesting their involvement in some retention. Nevertheless, in this case, the individual omission of each retentate did not modify the headspace concentration of this compound. But, in the same way as for 3-methylbutanol, this eventual contribution would be negligible in comparison with that of the mineral salts.

The studied volatile compounds were selected from the main aroma compounds of the Camembert cheese, and their concentrations were chosen in order to give sufficient peak area, but also to be as close as possible to their actual concentration in the cheese according to Molimard (1994). It is then conceivable that the water-soluble fraction components contribute, together with fat and proteins, to modulate both release and retention of volatile compounds, resulting in the whole cheese aroma.

3.2. Compensatory effects between the MWSE components

As shown above, components of the MWSE may have antagonistic effects on the release of some aroma compounds. Whereas some non-volatile components may lead to retention of a volatile compound, others may increase its release. The opposite effects, when they cause a non-significant global impact of the non-volatile WSE, were defined as compensatory effects. Thus, even if many of the aroma compounds previously studied were not affected by the presence of the global MWSE, it seemed interesting to investigate the results of omission tests on them. Thereby, it might be possible to identify eventual compensatory effects. For this purpose the influence of amino-acids, minerals and peptides were studied using omission tests. In contrast to omission of peptides and minerals whose results are given in Table 4, amino acids did not cause any change in the headspace concentration of the volatile compounds considered. Consequently, these data were not presented.

3.2.1. 2-nonanol

The existence of opposite contributions of the MWSE components on the volatile fraction is highlighted in the case of 2-nonanol (Table 4). Whereas the omission of minerals caused a significant retention of 33% ($P<0.001$), the omission of peptides was responsible for a significant release of 109% ($P<0.001$). Consequently, antagonistic effects of minerals (release) and peptides (retention) may allow the absence of MWSE effect to be explained. According to several workers, the existence of hydrophobic bonds between protein material and aroma compounds would favour retention phenomena (Bakker, 1995; Guichard & Langourieux, 2000; Reiners et al., 2000). Langourieux and Crouzet (1995) showed that β-lactoglobulin, which is the main water-soluble protein in the whey, interacts with small hydrophobic molecules. Moreover, the hydrophobic pocket of the protein may be involved in hydrophobic bonds with ketones and alcohols according to Pelletier et al. (1998). However, β-lactoglobulin (18 kDa) and the other water-soluble proteins are only present at trace levels in the cheese and subsequently in its WSE. Otherwise, it would have been possible to find some proteins or big peptides in the WSE, which might be present in RUF10. The use of an HPLC-electrospray-MS allowed for the RUF10 mass distribution to be known (unpublished results). The higher molecular weight encountered in this fraction was lower than 5 kDa. Thus, even if the RUF10 contained some proteins, they would be present at a trace level. Consequently, hydrophobic bonds with proteins were certainly not the main phenomenon occurring in the present study. Apparently, no interaction phenomenon between peptides and aroma compounds has been cited in the literature. In order to have more information about the non-volatile protagonists involved in the concerned retention, retentates were omitted separately. As none of the peptide fractions allowed for a significant retention to be shown, further

Table 4
Effects of salts and peptides on aroma compounds[a]

Aroma compounds	Salts	Totality of peptides	RUF10	RUF1	RNF
2-undecanone		Release*	Release*	Release**	Release**
2-nonanol	Release***	Retention***			
2,4-dithiapentane		Release**			
Ethylhexanoate	Release*				

[a] RUF10, RUF1, RNF: Retentates obtained, respectively, by 10 kDa cutoff and 1 kDa cutoff ultrafiltration then 0.5 kDa nanofiltration of the water-soluble extract of Camembert cheese.

* $P<0.05$

** $P<0.01$

*** $P<0.001$

studies would be necessary to specify the nature of the retention phenomena. Nevertheless, their contribution, in an additive way, to the whole retention effect measured for peptides, appears as probable.

3.2.2. 2-undecanone

In contrast to the case of 2-nonanol, peptides were shown to be responsible for a significant release of 2-undecanone because, when they are omitted, a decrease of its headspace concentration (37%, $P<0.05$) was observed. As WSE peptides have been shown to be small molecules, they may be involved as are sugars (Voilley et al., 1977) or nucleotides (Schinneller et al., 1972) in a "salting-out"-like phenomenon. Successive omissions performed with each fraction of peptides (RUF10, RUF1 and RNF) allowed for their respective roles to be specified. Thus, significant decreases of 2-undecanone headspace concentration of, respectively, 9% ($P<0.05$), 39% ($P<0.01$) and 77% ($P<0.01$) were observed when RUF10, RUF1 and RNF were omitted. So, they seemed to be all involved in the release caused by the whole peptide fraction omission. This demonstrated an additive contribution of each peptide fraction in the phenomenon observed with the sum of them. To confirm the opposite effect of peptides on 2-nonanol and 2-undecanone, further investigations would be necessary.

Additionally, since we did not observe any interaction between the total MWSE and this aroma compound, other MWSE components such as amino acids or salts, may have an opposite effect, cancelling the influence of peptides. Respective omissions of amino acids and salts did not show any significant effect. Nevertheless, it may be hypothesised that each of them, together with other MWSE components, will contribute, in mixture, to the retention of 2-undecanone in an additive way. These groups of compounds might indeed generate a global retention sufficient to cancel the release effect of peptides.

3.2.3. Ethylhexanoate and 2,4-dithiapentane

As for the two previous compounds, the total MWSE was not shown to have any effect on the headspace concentrations of either ethylhexanoate or 2,4-dithiapentane. In the case of ethylhexanoate, a significant release effect ($P<0.05$) of the minerals was shown by the decrease of its headspace concentration (28%) with their omission (Table 4). As no global MWSE effect was demonstrated, some other non-volatile water-soluble compounds probably generate a retention phenomenon to counterbalance the salting-out effect due to minerals. With 2,4-dithiapentane, the omission of peptides was responsible for a significant retention (29%, $P<0.01$). So, the presence of peptides leads to a release of this compound and we can hypothesise that an additive retention by other compounds may then occur to explain the absence of a MWSE effect.

4. Conclusions

Among the volatiles studied, only three seemed to be affected by the presence of the MWSE. When they contributed to the headspace composition, mineral salts were shown to have a releasing effect on any aroma compound. A salting-out phenomenon may give a good explanation for such an observation. On the other hand, peptides may take part either in retention or in release. Thus, by modifying the composition of non-volatile WSE components, it might then be possible to modulate the flavour of Camembert cheese. For instance, by altering the formulation, it may be possible to displace the flavour balance, thus changing the relative weight of eventual opposite effects. Such changes may account for the change in cheese flavour during ripening by modification in the WSE composition. However, to this end, sensory analysis may be necessary to actually evaluate these physico-chemical interactions and the influence of water-soluble components on the flavour. In order to demonstrate the importance of interactions between aroma compounds and non-volatile water-soluble molecules in the whole cheese context, the relative impacts of WSE, fat and proteins will have to be investigated.

Finally, the existence of such physicochemical compensatory phenomena underlines the importance of omission tests to reveal relative contributions in the context of a complex mixture. In order to specify the molecular nature of these interactions, further fundamental studies will be necessary.

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