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Effect of the temperature on the release of aroma compounds and on the rheological behaviour of model dairy custard

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Abstract

The influence of temperature was pointed out on the release of aroma compounds from a model dairy dessert and on its rheological behaviour. The recipe chosen for inter-laboratory studies within the programme of COST Action 921 was tested. The custards were flavoured with three aroma compounds belonging to strawberry note. The partition coefficients of three compounds (ethyl butyrate, ethyl hexanoate, *cis*-3-hexenol) were determined at three temperatures (12 °C: tasting temperature of refrigerated dairy products, 20 °C: room temperature, and 37 °C: mouth temperature). Two textures of the custards were obtained using kappa- and iota-carrageenan. Penetrometry tests characterized the rheological properties of the custards. Whatever the temperature, aroma compounds had a greater affinity for the custard rather than for the water. Moreover, the temperature modified the rigidity character of the custard, which should affect the repartition of the aroma compounds between vapour phase and custards.

Keywords: Temperature; Release; Aroma compounds; Rheological behaviour; Custard

1. Introduction

Most food products are complex in terms of chemical composition and structure: they are often multi-component materials and present several phases. Many factors influence the partition of the aroma compounds between the different phases of the food matrices, their release and their perception. The release of aroma compounds from food matrices is mainly governed, on the one hand by the physicochemical interactions between aroma compounds and the food constituents of the matrices (Kinsella, 1989), and on the other hand by the structural characteristics of the food matrix (Cayot, Pretot, Doublier, Meunier, & Guichard, 2004). The release and its rate of transfer from one environment to another depend on temperature, food composition, structure, viscosity of the different phases, molecular interactions (with proteins, lipids, carbohydrates) and on reversible (hydrophobic interactions between aroma compounds such as esters and proteins) and non-reversible binding (such as covalent binding of t-2-hexanal with dairy proteins (Meynier, Garillon, Lethuaut, & Genot, 2003)). The distribution of an aroma compound within a food depends on its affinity for these different phases and its ability to release in the vapour phase. The nature of the different non-volatile constituents such as proteins, lipids, carbohydrates, and salts has a great impact on the retention of the aroma compounds by the food matrices (Bakker, 1995; Guichard, 2002). Many studies dealt with simple systems containing either protein solutions, the most studied protein being β-lactoglobulin (Fischer & Widder, 1997; Guichard & Langourieux, 2000; Seuvre, Espinosa Diaz, & Voilley, 2001; Reiners, Nicklaus, &

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Guichard, 2000), or lipids (De Roos, 1997; van Ruth, King, & Giannouli, 2002) or carbohydrates (Escher, Nuessli, & Conde-Petit, 2000; Godshall, 1997; Goubet, Le Quere, & Voilley, 1998). Physicochemical interactions could take place between aroma compounds and food constituents. These interactions could modify the repartition of these aroma compounds inside the food. The retention and/or the release of the aroma compounds were influenced and thus their sensorial perception. Nevertheless few works have reported on the behaviour of aroma compounds in complex media (Seuvre, Philippe, Rochard, & Voilley, 2006).

This work contributes to the program of COST Action 921 whose goal is to study the structural organization of food matrices and its impact on flavour release and perception. The chosen matrix is a custard dessert containing polysaccharides (tapioca starch and carrageenans, the latter having a synergistic effect with starch and proteins on texture (Cayot, 2006)) and milk. The custard is flavoured with aroma compounds belonging to strawberry note. The aim is to study the influence of temperature on the release of the aroma compounds and on its rheological behaviour.

2. Experimental

2.1. Materials

2.1.1. Aroma compounds

Three aroma compounds (ethyl butyrate, ethyl hexanoate, *cis*-3-hexenol (hex-3-en-1-ol)) belonging to the strawberry aroma with various physicochemical properties were chosen and are given in the Table 1.

2.1.2. Preparation of the custard

2.1.2.1. Composition. The recipe of the food matrix was composed of 500 mL of whole-fat milk (UHT, 3.6% fat), 36.55 g of commercial sucrose, 0.1125 g of carrageenan (kappa-carrageenan supplied by Rhodia, France, or iota-carrageenan supplied by Cargill Texturizing Solutions) and 23.5 g of modified tapioca starch VA60T (kindly provided by AVEBE for COST Action 921).

2.1.2.2. Preparation procedure of the custard. Whole-fat milk was introduced under stirring (132 rpm) in a flask (stainless steel container) placed in an oil bath previously pre-heated at 40 °C. The powders (starch, sucrose and carrageenan) were weighed and slowly dispersed in milk (during 4 min); the mixture was stirred continuously. The hydration of the powders in the mixture was continued during 10 min at 40 °C under stirring. Then the bath temperature was increased until 95 °C (2.5 °C/min). When the custard temperature reached 90 °C, this final temperature (90 °C) was maintained 10 min. After the heating process, the stainless steel flask containing the mixture was cooled in a fresh water bath under stirring (132 rpm) in order to cool down the preparation until we reached 40 °C. At this temperature flavour was added continuing the stirring 1 min more to ensure its dispersion. Then the custard was sampled and introduced in tight flasks and stored at 4 °C for 3 days before analysis. This delay was applied in order to equilibrate the aroma with the custard.

2.2. Methods

2.2.1. Flavour partitioning

2.2.1.1. Headspace analysis. Samples of 60 g of aromatised (ethyl butyrate: 240 mg/kg, ethyl hexanoate: 50 mg/kg and *cis*-3-hexenol: 40 mg/kg) custard (recipe COST with kappa-carrageenan), or water taken as reference, were introduced in the thermostated flasks (12, 20 and 37 °C). This flask was composed of a cylindrical glass (40 mm diameter by 92 mm height) equipped with a thermostated doublewall. Then the flasks were obturated with Teflon® cork and in incubators at 12, 30 or 37 ± 0.5 °C. Preliminary experiments at different times were used to ensure that the equilibrium was reached after 2 h. Then a flow (36.2 mL/min) of inert gas (N_2) passed through the flask (t_0 = beginning of N_2 flow) in experiment. The first automatic injection of headspace samples (1 mL) into gas chromatograph was realized at 1.5 min and after each 30 min. The injections were done until the exhaustion of the vapour phase.

2.2.1.2. Chromatographic analysis conditions. Quantitative analysis of the aroma compounds were done using Chrompack CP 9000 chromatograph equipped with FID detector (200 °C), with 190 °C in the injector and 90–110 °C in the column (stainless steel column 3 m length, 2.2 mm inner diameter, packed with Chromosorb W-AW 80-100 mesh Carbowax 20M-10%). Operating conditions were as follows: N_2 carrier flow rate: 36.2 mL/min, H_2 flow rate: 33.7 mL/min and air flow rate: 257.8 mL/min. Each experiment was carried out at least three times using three different batches of matrix.

2.2.1.3. Determination of mass partition coefficient (K_{mass}). The mass partition coefficient between gaseous phase and matrix K_{mass} was determined at three temperatures (12, 20 and 37 °C) using the relation: $K_{\mathrm{mass}} = C_{\mathrm{v}}/C_{\mathrm{m}}$ with C_{v} and C_{m} the flavour mass fractions (w/w) in the vapour and in the matrix phase, respectively.

2.2.2. Rheological characterization

The penetrometry tests were performed with the help of a texture analyser RHEO TA-XT2 (Champlan, France). The samples of 60 g of aromatised custard stored in polypropylene boxes at 4 °C were reconditioned at the chosen temperatures (12, 30 and 30 °C) during 2 h and were put directly onto the platform of the texture analyser.

Penetrometry measurements at a constant rate (1 mm/s) were done using a 25 mm diameter cylinder. Penetration was continued down to 30% of the initial height of the sample. Then the cylinder returned to its initial position with a 10 mm/s rate. The cylinder was cleaned after each measure. The registration began with the measurement of the force

Table 1
Physicochemical properties of the aroma compounds

Aroma compounds	Molecular weight (g/mol)	Molar volume[a] (cm^3/mol)	Saturated vapour pressure[b] (Pa) at 37 °C	Water solubility[c] (g/L) at 37 °C	Hydrophobicity (log P)[d] at 25 °C
Ethyl butyrate ($C_6H_{12}O_2$)	116.16	151	3839	5.3	1.73
Ethyl hexanoate ($C_8H_{12}O_2$)	144.21	196	500	0.52	2.79
cis-3-Hexenol ($C_8H_{12}O$)	100.16	129	317	14.7	1.34

[a] Estimated data, at 25 °C, Tyn and Calus method, 1975.
[b] Estimated data, software MPBP.WIN, 1994–1997.
[c] Experimental data, Philippe et al. (2003).
[d] Estimated data, Hansch and Leo (1979).

which increased until $F_{max}$when the probe was going down into the sample. Then the force decreased when the probe was going back to its initial position and a negative force was measured.

Four parameters can be extracted from the recording measurements of the force vs. time curves: the maximum force on the curve F_{max} (N), the maximum of negative force F'_{max}, the positive area (N/s) corresponding to the stiffness characteristics of the product, and the negative area (N/s) corresponding to the sticking characteristics of the custard (Arvisenet & Cayot, 2001; Cayot, 2006). Each experiment was conducted 10 times.

2.2.3. Statistical analysis

The data were analysed and compared by using variance analysis and Student–Newmans–Keuls' test ($\alpha = 0.05$) to determine the significance of differences, on SAS software (SAS Institute Inc., version 9.1., Cary, NC).

3. Results and discussion

3.1. Comparison of the mass partition coefficients in pure water and in custard

The results are given in Table 2.

In water the mass partition coefficients increased with temperature: a greater quantity of aroma was released from water which indicated that the physicochemical interactions between aroma compounds and water were weak because a small increase of temperature was able to broken them partially. Because ethyl butyrate and ethyl hexanoate were the most hydrophobic compounds ($\log P = 1.73$ and 2.79, respectively, Table 1), they had less affinity for water molecules and had a tendency to escape from water; moreover, the increase of temperature induced an increase of the agitation of the molecules and favoured their release. The most hydrophilic molecule, *cis*-3-hexenol with the higher water solubility (Table 1), presented a hydroxyl group which was able to interact with water molecules by hydrogen bonding. With the increase of temperature, this kind of interactions was gradually broken and this aroma compound was all the more released.

In custard, the same behaviour with the increase of temperature was observed but with lower values than in water that indicated a greater affinity of the aroma compounds for the custard. Seeing that custard was a complex medium with lipid, milk protein, starch and carrageenan, these results were expected. However, in the two media, water and custard, the intensity of the interactions were weak since they were broken with the increase of temperature. The aroma compounds were able to establish interactions of different natures and were more numerous in custard than in water, which explained the differences of retention.

Whatever the temperature, the three aroma compounds showed a higher mass partition coefficient in water than in custard indicating a greater retention in the custard. The differences of release of each aroma compound from water and custard at a given temperature were estimated by the calculation of the K_{mass} variation (Table 3). Ethyl hexanoate exhibited the highest K_{mass} variations (>95%) for each temperature (the most hydrophobic and the less water soluble compound) and *cis*-3-hexenol (the less hydrophobic and the most water soluble compound) showed limited K_{mass} variations (45–60%).

To compare the influence of the temperature on the release of the aroma compounds in the two media (water and custard), $\ln K_{mass}$ was plotted in function of the inverse of temperature (Fig. 1). Two compounds, ethyl hexanoate

Table 2
Mass partition coefficients in pure water and in custard cream at 12, 20 and 37 °C

K_{mass}	Pure water			Custard cream		
	12 °C	20 °C	37 °C	12 °C	20 °C	37 °C
Ethyl butyrate	2.25 ± 0.13	5.34 ± 0.11	11.65 ± 1.16	0.81 ± 0.05	1.38 ± 0.08	2.61 ± 0.26
Ethyl hexanoate	3.32 ± 0.20	7.55 ± 0.45	16.51 ± 1.65	0.12 ± 0.01	0.22 ± 0.01	0.61 ± 0.06
cis-3-Hexenol	0.11 ± 0.01	0.49 ± 0.03	1.50 ± 0.15	0.06 ± 0.01	0.20 ± 0.01	0.68 ± 0.07

Table 3
Variation[a] of release (%) of aroma compounds in water and in custard cream in function of temperature

	12 °C	20 °C	37 °C
Ethyl butyrate	64 ± 4	74 ± 2	77 ± 4
Ethyl hexanoate	96 ± 1	97 ± 1	96 ± 7
cis-3-Hexenol	45 ± 5	59 ± 5	54 ± 9

[a] Variation = [(K_{mass} in water − K_{mass} in custard cream)/K_{mass} in water] × 100 at a given temperature.

and *cis*-3-hexenol presented a similar behaviour with slopes in the same range of values. But in the case of ethyl butyrate the slope was lower in custard than in water. The energy brought by the increase in temperature broke less binding in the custard than in water. For the three aroma compounds, the values of the origin ordinates were lower in the custard indicating a weaker volatility in this media. The aroma compounds were more retained in custard because the interactions could be of hydrophobic nature and this kind of interactions was less influenced by the temperature than hydrogen bonding.

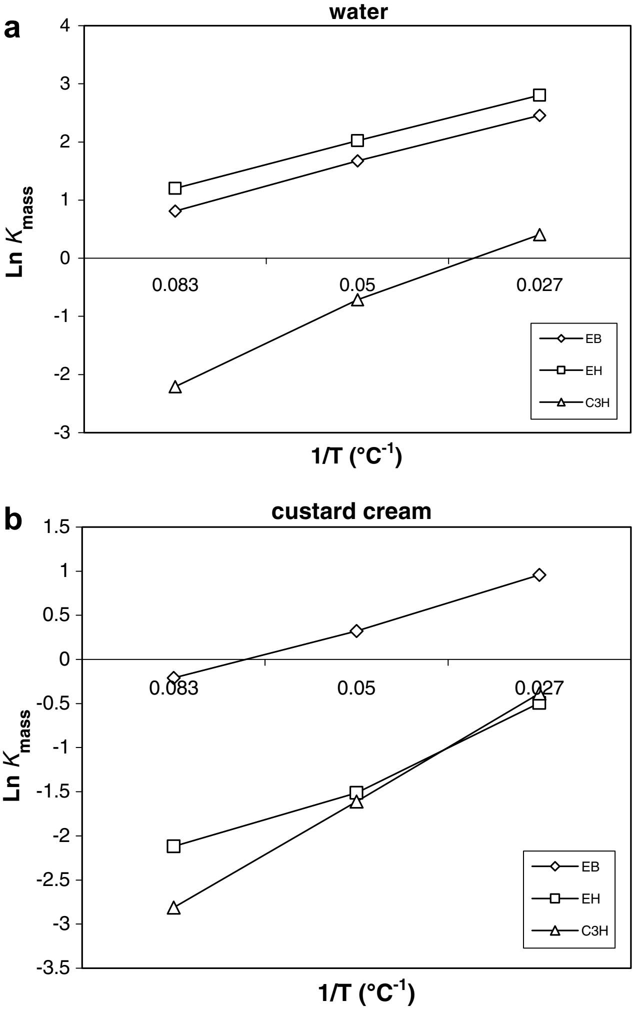

Fig. 1. Influence of the temperature on the release of aroma compounds.

The enthalpies of vapourisation of the aroma compounds were calculated (from Fig. 1) in water and in custard (Table 4). The relationships ($\ln K_{mass} = f(1/RT)$) were linear (Fig. 1), which indicated that in this range of temperatures (12–37 °C), the enthalpies of vapourisation can be considered as constant. For ethyl butyrate, the enthalpy of vapourisation ΔHv in pure water was higher than ΔHv in custard: therefore the interactions "ethyl butyrate–water" were more difficult to break than the interactions "ethyl butyrate–custard" (containing fat). The kind of interactions in the two media seemed to be different. This behaviour was already observed for other esters: their enthalpies of vapourisation where higher in anhydrous milk fat than in water and skim milk (Meynier et al., 2003).

For ethyl hexanoate, the interactions "ethyl hexanoate–water" were of the same intensity than interactions "ethyl hexanoate–custard" since ΔHv in water was in the same order than ΔHv in custard. Similar behaviour was observed for *cis*-3-hexenol: the interactions "*cis*-3-hexenol–water" had the same intensity as the interactions "*cis*-3-hexenol–custard" (ΔHv water ~ ΔHv custard).

The enthalpies of vapourisation ΔHv did not follow the same variations as the partition coefficients K_{mass}. The partition coefficients takes into account the vapour pressure of the compound as well as the environment of the aroma compound when the enthalpies are linked to the attraction of repulsion forces that retain or release the aroma compounds in their environment (Meynier et al., 2003). The values of ΔHv in water compared to that in custard cream depended on the aroma compounds. All the K_{mass} values in water were higher than in custard cream that showed higher retentions in the custard cream than in water whatever the temperature.

3.2. Rheological behaviour

The gelling types of iota- and kappa-carrageenan are widely used in a large variety of food, imparting textures from soft and elastic to rigid and brittle. The four parameters extracted from the recording measurements of the force vs. time curves were determined at three temperatures (12, 20 and 37 °C) and for three different preparations of custard (with kappa- or iota-carrageenan and without carrageenan).

Table 4
Enthalpies of vapourisation of pure aroma compounds, aroma compounds in water and in custard cream

Flavour	Pure compound ΔHv (kJ/mol)	In pure water ΔHv (kJ/mol)	In custard ΔHv (kJ/mol)	Variation ΔHv (%)[a]
Ethyl butyrate	41.8	46.7	33.9	−27
Ethyl hexanoate	50.1	45.5	47.5	4 (NS)
cis-3-Hexenol	57.7	72.3	68.6	−5 (NS)

NS: No significant.
[a] Variation ΔHv = [(ΔHv in pure water − ΔHv in custard)/ΔHv in pure water] × 100.

3.2.1. Maximum force F_{max} (N)

Fig. 2a showed the compressive force necessary to penetrate 30% of the initial height of the matrices. The two matrices containing carrageenans (kappa or iota) presented a similar behaviour with the highest force F_{max} registered at 12 °C and the lowest force F_{max} at 37 °C. Nevertheless F_{max} values in iota-carrageenan were ever higher than those in kappa-carrageenan. For these two matrices the behaviours at the three temperatures were significantly different. The matrix without carrageenan showed lower F_{max} values with a lesser impact of the temperature. These results are in agreement with the study of Verbeken, Bael, Thas, and Dewettinck (2006): they pointed out that an attractive interaction took place between kappa-carrageenan molecules and milk proteins in sterilized dairy desserts. They added that, due to this interaction, milk proteins were involved in the formation of the carrageenan gel network and contributed to the physicochemical properties of the desserts.

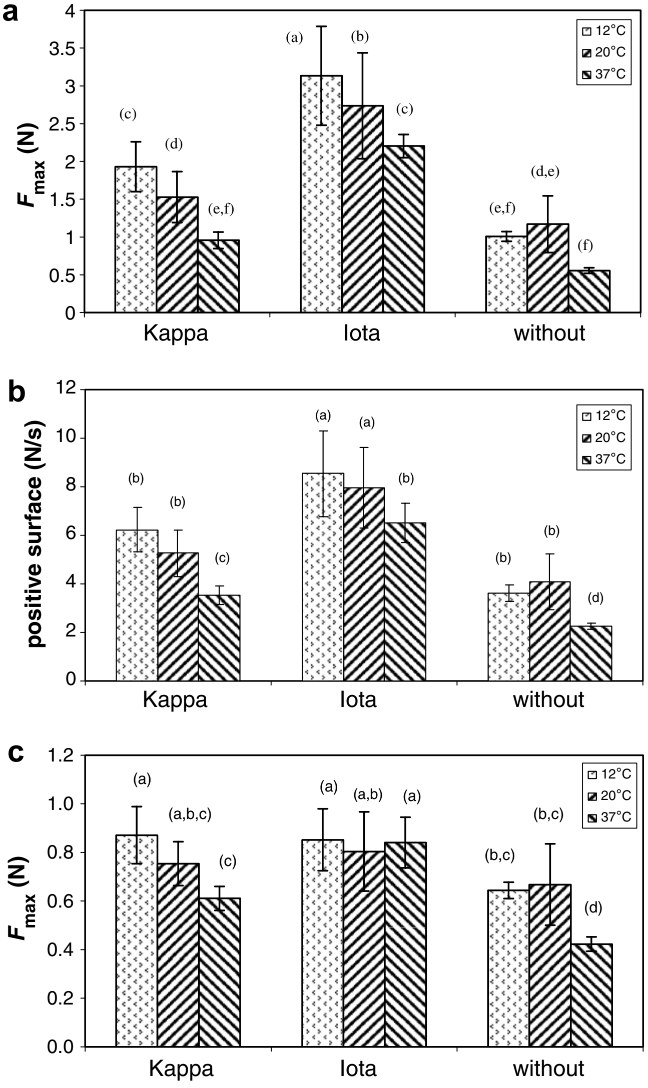

Fig. 2. Influence of the temperature on the rheological behaviour of the custard cream. (a) Maximum force F_{max} (N). The values with a different letter (a–f) are significantly different at a level 5%. (b) Positive surface (N/s). The values with a different letter (a–d) are significantly different at a level 5%. (c) Maximum force F'_{max}. The values with a different letter (a–d) are significantly different at a level 5%.

3.2.2. Positive area (N/s)

This parameter defined by the area under the force deformation curve registered the resistance to the penetration of the cylinder into the matrix and corresponded to the stiffness characteristics of the product. Fig. 2b showed the influence of the nature of carrageenan and the influence of the temperature on this parameter. This Fig. 2b reminded us of the previous one (Fig. 2a): kappa- and iota-carrageenan induced differences of stiffness with higher values registered in iota-carrageenan. The increase of temperature (from 12 to 37 °C) produced a significant decrease of stiffness even in absence of carrageenan. However, no significant difference was observed between 12 and 20 °C, perhaps because the variation in temperature is not important enough to record a significant difference.

3.2.3. Maximum force F'_{max}

This parameter corresponded to the force necessary to detach from the cylinder of the matrix. Fig. 2c shows that this parameter was influenced by the increase of temperature (12–37 °C) only with the matrices prepared with kappa-carrageenan or without carrageenan.

3.2.4. Negative area (N/s)

This last parameter extracted from the recording measurements of the force vs. time curves expressed the resistance to the withdrawal of the cylinder and corresponded to the sticking characteristics of the custard. No significant differences were registered due to variations of temperature or variations in the composition of the matrices on this parameter.

Fig. 2a and b showed that temperature influenced stiffness characteristics of the matrices. These two macromolecules, iota- and kappa-carrageenan, differ only by the presence of a sulphate group on galactose residue. The galactose residues have an oxygen bridge between carbons 3 and 6. Thanks to it, the kappa and the iota-carrageenans can form a double helix in the presence of cations (K^+ and Ca^{2+} present in milk preparations) and this enables them to create a gel which is formed during the cooling of the solution. The K^+ ions are necessary to gelation and the Ca^{2+} ions increase the rigidity of gel. The helixes are stabilized by the presence of water molecules which form hydrogen bonds between the double helixes (Piculell, 1995). Carrageenan gelation is importantly affected by the presence of other biopolymers. In gelled dairy desserts of the pudding or custard type, carrageenan gelation is typically influenced by the presence of starch, used as thickening agent, and milk proteins (Verbeken, Thas, & Dewettinck, 2004). The increase of the temperature involved a reduction in the rigidity of the matrices, whereas the sticking character did not seem affected. The increase of temperature undoubtedly affected the hydrogen bonds or the interactions with low energy but did not act on ionic interactions. The increase of temperature involved a modification of the rheological behaviour of the gel by inducing a more soft gel texture. The chains would maintain their aptitude to form structures in double helix but perhaps with a modification of the rate of helicity.

Texture modification involves differences in perception. Pangborn and Szczesniak (1974) showed that the addition of polyosides in water involved a reduction in the perception of all compounds of aroma selected in their study. Moreover, perception of the flavour and odour decreased more especially as the thickening agent concentration increased: the low polarity and the great volatility of the aroma compounds made it possible to explain the reduction in the release of composed by the presence of polyoside.

Recent studies have shown that the variation of the textural agent in dairy desserts modulated the intensity of the fruity aroma. The intensity of the aroma was higher in soft dairy composed of λ-carrageenan, assessed as the most unctuous dairy desserts (Lethuaut et al., 2005). This work concluded that, beside sweetness assessment, textural characteristics had an effect on the aroma assessment.

In addition, Juteau, Tournier, and Guichard (2004) showed that there were differences in perception for certain aroma compounds between samples of different textures. These differences depended on the nature of the compounds, on the nature of the polyosidic matrix and on the sensory method used (with the nose or in mouth). In the case of gelled system, when the hardness of the gel increases under the effect of the increase in the concentration in gelling agent, the compounds of flavour are released in the headspace and the perceived flavour is less intense. The presence of a three-dimensional network within the matrix slows down the migration of the volatile compounds towards the interface matrix–air (Juteau et al., 2004).

The increase of temperature involving a decrease of the rigidity of the gels, the migration of the aroma compounds would be less reduced.

4. Conclusions

Indeed, the release of the aroma compounds depends of their physicochemical characteristics and of the matrix but also of the temperature. Each aroma compound had its own behaviour towards the matrix at a given temperature. The matrix also acted on the release of aroma compounds: the custard contained molecules, in particular lipids, which had strong interactions with the aroma compounds and prevented their release.

The temperature influenced the release: an increase of the temperature involved an increase in the release of the aroma compounds but in different proportions for each compound. Whatever the temperature aroma compounds had a greater affinity for the custard than for water. Moreover, the temperature modified the rigidity character of the custard which should affect the repartition of the aroma compounds between vapour phase and custards.

The temperature induced two modifications: on the one hand it modified the partition coefficients matrix/air of the aroma compounds and on the other hand the rigidity of the matrix. These two factors will disturb the interactions of low energy. The flavour of a food will thus be different according to the tasting temperature.

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