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Changes in aromatic volatile composition of strawberry after high pressure treatment

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Abstract

The aromatic volatile compounds of high pressure treated strawberry coulis (*Fragaria ananassa* Gariguette) were analysed by capillary gas chromatography–mass spectrometry (GC–MS) and compared with aromatic volatile compounds of raw strawberry and heat-treated strawberry coulis. Characterisation of treated and untreated samples was achieved by applying principal component analysis to the chromatographic data. Aroma of strawberry was specifically identified by furaneol (2,5-dimethyl-4-hydroxy-furan-3-one) and nerolidol (3,7,11-trimethyl 1,6,10-dodecatrien-3-ol). No significant changes of all the aromatic volatile compounds were observed between untreated and high pressure-treated (200 and 500 MPa, 20° C, 20 min) strawberry coulis. On the other hand, changes appeared in the composition of aromatic compounds after an ultra high hydrostatic pressure treatment at 800 MPa (20 min, 20° C) and after a sterilisation (120° C, 20 min). (© 1999 Elsevier Science Ltd. All rights reserved.

1. Introduction

The strawberry is a classic example of a sought-after quality fruit that is, unfortunately, also highly perishable. Ensuring that top quality strawberries reach the consumer in perfect condition requires very careful attention to their distribution, storage and final display in the shops. Fresh strawberries have a very short life because they are easily bruised and the fact that 'if nothing is done' they quickly succumb to fungal attack, principally from *Botryris cinerea* (Guichard, Chambroy, Reich, Fournier & Souty, 1992). At the moment those cultivars with exceptional flavour are sold at a premium for fresh fruit distribution but this quality differential for fresh fruit becomes less marked after the fruit has been subjected to traditional methods of processing. Because of this, the producers (and potential processors) of high quality strawberries are interested in any new process that could help to extend the range of processed food products if the quality differential with respect to other cultivars is maintained. Such a process would retain the organoleptic properties of the raw fruit

(that is its texture, colour, taste and smell) as well as the vitamins. The quality, then, would be irreproachable and equivalent to that of the raw fruit though not necessarily presenting the same aspect. Market research has shown that there is a marked demand for high quality fruit components as ingredients in processed food products. These products could be in the form of coulis, sauce, jam or other products that might, for example, form part of a processed milk or dessert preparation.

The traditional process for stabilising and conserving foodstuffs has been the thermal process and its use in modern food processing has relied on increasingly higher temperatures. One of the consequences of this is that not only does the texture and flavour of the foodstuff suffer but the vitamins and smell can be destroyed and the damage can even extend to the production of toxic compounds (Hayashi, 1995). In contrast to this, the use of high pressure for stabilising and conserving foodstuffs retains the flavour (Cheftel, 1995), the colour, the taste of the natural foodstuffs (Butz, Koller, Tauscher & Wolf, 1994) and the natural properties of the products (Balny, Heremans & Masson, 1992). It would appear, therefore, that high-pressure technology could become a powerful tool for taking advantage of the opportunities offered by the 'top of the range' quality fruits as represented by certain cultivars of strawberries.

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The aim of this study has been to examine what effect high-pressure treatment has on the quality of premium strawberry fruit as measured by the incidence and concentration of the various volatile compounds present in the aromatic profile of a strawberry coulis after it has undergone a high-pressure treatment. The cultivar chosen was 'Gariguette' which is highly prized for its flavour. The analysis of the volatile aromatic releases was carried out using a gas chromatograph coupled with a mass spectrometer (GC-MS). The effect of different pressures was measured by comparing the results from using three different pressures (200, 500 and 800 MPa) for 20 min at ambient temperature. In order to be able to compare and contrast the results with those of the traditional sterilising process, a sample of coulis that had been sterilised at high temperature (120°C for 20 min) was also analysed by the same method. Lastly, an untreated coulis sample was used as a bench-mark reference for fresh fruit.

This study was conducted in two stages. The first one was qualitative and relied on a visual comparison of the various aromatic profiles together with a presentation of their constituent volatile compounds. In the second stage a quantitative analysis of the concentrations of the volatile compounds was undertaken and the relative importance of each family of volatile compounds compared for each of the three selected pressures and also for the sterilised sample. Lastly, an evaluation was made to see whether the variations between samples in the concentrations of the volatile compounds were statistically significant.

2. Materials and methods

2.1. Preparation of the samples

The variety of strawberries used was 'Gariguette', obtained from the Périgord region of France. The strawberries were obtained from normal retail outlets and were in prime condition. They were washed and detached from the stalk and then homogenised to the consistency of a coulis. No additives of any kind were used in the preparation of the coulis. Its physical and chemical characteristics are detailed in Table 1.

Each sample of coulis was sealed in a plastic bag (Cryovac-NOP-120) that could remain pliable, be

Table 1

Physical and chemical characteristics of the strawberry (var. Gariguette) coulis

Characteristic	Strawberry coulis
pH Water activity Refractometry index (degree Brix)	$\begin{array}{c} 3.64 \pm 0.026 \\ 0.975 \pm 0.002 \\ 8.7 \pm 0.1 \end{array}$

thermally sealed and was compatible with foodstuffs. Each sealing took 4 s at 100°C and was carried out at 80% vacuum to ensure that the minimum amount of air remained in the bag. The mass of each sample averaged 451.2 ± 2.31 g.

2.2. Brix degree

The measurement of the sugar content in degrees Brix was carried out using a hand-held refractometer (Model 1190). The refractometer was calibrated with distilled water and a zero reference obtained from the reading at 20°C. The instrument was accurate to 0.1 degree Brix. Each reading was repeated four times and the average recorded.

2.3. Water activity (a_w)

Water activity was measured by means of a GBX AW meter. Each reading was repeated four times and the average recorded. The instrument was accurate to 0.001. Calibration was carried out with saturated NaCl, with a defined water activity of 0.755 and with saturated KCl with a defined water activity of 0.432.

2.4. pH measurement

The pH value of the coulis was measured using a pHmeter (Model Accumet 15) accurate to 0.002 unit of pH. Calibration was carried out by reference to buffer samples of 4 and 7 pH. Each reading of a sample was repeated three times and the average recorded.

2.5. High pressure procedure

The hyperbar apparatus used was designed and produced by a partnership of NFM-Technologies (Le Creusot, France) and Framatome (Paris, France) to work at up to 800 MPa. It is being commercialised by Clextral (Firminy, France). The samples were placed in the 3-litre compression chamber and subjected to hydrostatic pressures of either 200, 500 or 800 MPa for 20 min at the ambient temperature with a pressureincreasing rate of 375 MPa per min. Both temperature and pressure were continuously monitored and their values recorded throughout each process. The pressure fluid used was water. Because of the adiabatic phenomenon, the 'in situ' temperature inside the compression chamber was higher than the monitored readings of 20°C. Using theoretical data on the thermodynamic properties of water under high pressure, the likely actual temperature with a pressure build-up to 400 MPa would be 31°C when water is used as the pressure fluid (Makita, 1992). However, this effect depends on the conductivity of the metal used for the high pressure vessel and the corresponding thermal transfer from the

water. The result is a temperature increase from 5 to 12° C (for pressurisation up to 800 MPa).

2.6. Sterilised samples

Certain samples were sterilised in order to make a comparison between the results of sterilisation and the results of the high-pressure process. Sterilisation of these samples was carried out in a water-steam vertical autoclave, model Apave, type 40×70 , for 20 min at 120° C, reaching a final pressure of 1.5 atmospheres.

2.7. Internal standard

Tridecane was selected as the internal standard. It was chosen because of the fact that it would mask none of the constituent peaks of the gas chromatography profile (Gunther-Douillard, 1988). A solution of this internal standard was prepared in ethanol in the proportion 20 μ l tridecane to 50 ml ethanol so that, if 500 μ l of the solution were added to 150 g of the coulis, the concentration of tridecane in the fruit would be approximately 1 mg/kg of fruit.

2.8. Extraction of the volatile compounds

After the completion of both the sterilisation and high-pressure processes the samples were frozen and stored at -18° C until required for the extraction of the volatile compounds. The maximum delay before extraction was 7 days.

The extraction was carried out by centrifuge once the samples had thawed out and was repeated three times for each of the five sample types (three pressure values, sterilised and untreated).

Thawing was achieved in 15 minutes and each sample $(149.7 \pm 5.94 \text{ g})$ of strawberry coulis was mixed with 100 ml of pure water obtained through a filter system (Millipores) where the water temperature was maintained at between 0 and 5°C. The suspension was then transferred to an Erlen-Meyer flask of capacity 1000 ml and 50 ml of dichloromethane (previously purified by distillation) was added together with 500 (µl of the solution of the internal standard. The flask was placed in an ice bath and subjected to vibration in a magnetic field for 30 min. The resulting emulsion that formed in conjunction with the dichloromethane made it impossible to pour directly from the flask into the test tube. To recover the organic extract, the slurry was separated from the solids in suspension through the use of a centrifuge operating for 30 min at $5031 \times g$. The liquid extract was poured into a separate container and the final sediments were re-extracted with 10 ml of dichloromethane: this extract was pooled (Radford, Kawashima, Friedel, Pope, & Gianturco, 1974). The solution that formed was added to the slurry from the original centrifuge operation and

the remaining solid residue left behind thrown away. Two other extractions were carried out. The slurry with washings was then vibrated and centrifuged for 15 min each time and the process of extracting the organic extract repeated. The end result was 80 ml of liquid extract that was then concentrated twice, once with a Kuderna-Danish apparatus (Garnier, Quetigny, France) of 100 ml capacity and then a second time in a similar apparatus of 5 ml capacity. A third concentration was effected under a stream of nitrogen gas to produce finally 500 µl of concentrate.

2.9. Gas chromatography

Gas chromatography analysis of the total organic extract was carried out using a Hewlett Packard HP 5890 apparatus equipped with a splitless-split, associated with a septum purge activated at 250°C and adjusted to give a gas discharge vector of between 1 and 3 ml/min in order to avoid any danger of the gas components spoiling the results. The division valve was opened 20 s after beginning the injection, keeping to a discharge value of 20 ml/min. The gas chromatograph (GC) phase is polyethylene glycol treated with nitroterephthalic acid. A free fatty acid phase column (FFAP, J & W Scientific, Folsom, CA) was used because it is appropriate for the analysis of acids and phenols when the presentation of gaussian peaks is required. The column was 30 m long, internal diameter 0.32 mm, phase thickness 0.25 µm and rate of discharge 2 ml/min. Peak areas were recorded with a Hewlett Packard computer.

2.10. Mass spectrometry

The individual peaks from the chromatogram were registered and identified using the Hewlett Packard CPG/GPC mass spectrometer, MSD5970. This is a quadripole mass spectrometer used in electron impact mode. The electron energy was 70 eV and the temperatures for the source and interface were, respectively, 150 and 240°C.

The identification of the spectra obtained was achieved with the aid of the 'Wiley using X Windows' composition library, or other sources such as 'Eight Peak Index Spectra' (MSDC, 1974) and 'Compilation and Mass Spectra of Volatile Compounds in Foods (TNO)' (Noever, Bouwnan, Tasa, & LaVas, 1981). However, certain compounds remained unidentified.

2.11. Quantitative determination

The names of the quantified compounds are itemised in Table 2. Chromatographic response coefficients of aromatic volatile compounds were determined by using reference substances in known concentrations.

Table 2								
The volatile compounds	detected in	n the	strawberry	coulis	and	their	associated	odours

2.15Ethyl butanoateEthyl putanoate2.48Butyl acetateFula; cher-fuit, sour-chesse2.48Heytan-2oneFula; grass-fuit, unitopf fuit3.80Heptan-2oneForal, sergas-fuit, unitopf fuit3.80Melhyl hexanoateBinan, swetc-aprice furt4.49Cis-hexen-3-alBinan fuit, sour-chesse5.55Heyl acetateBinan fuit, sour-chesse5.57Heyl acetateMeinyl fuet6.00Tridecane (internal standard)Irige ran7.30Hexen-1-olGrass, green, oily fuit7.31Hexen-2-olUnipe fuit, unipe orange, foral8.13NonanalHoral-Fuit, unipe orange, foral8.14Hexen-2-olUnipe fuit9.84Lindool orydeIrige1.18Lindool orydeIrige1.18Lindool orydeIrige1.21J-Ethanediol diacetaeTotal, runi, lemon-woeded1.22J-Methyl prognomics acidRanci, runid butter, frait1.34Oryl butanosteGreen-grass, orange-floral, carthy1.40Hexinova Chevil plutano or 2.5Irige1.41Hydroxy methyl furan or 2.5Irige1.42Hexinova Chevil plutanoitStale-cheese, frait, ranid butter1.54Hexinova Chevil plutanoit acidRanci, runid butter1.54Hydroxy methyl furan or 2.5Irige1.54Hydroxy methyl furan or 2.5Irige1.54Hydroxy methyl furan or 2.5Irige1.54Hydroxy methyl furan or 2.5Irige <th>Retention time (min)</th> <th>Volatile compound</th> <th colspan="3" rowspan="2">Associated odour^a Ether-fruit, fruit, sour-cheese</th>	Retention time (min)	Volatile compound	Associated odour ^a Ether-fruit, fruit, sour-cheese		
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41.39 9,12-Octadecadienoic acid	40.57	Octadecadienoic acid			
	41.39	9,12-Octadecadienoic acid			

^a Gunther-Douillard (1988).

The percentages of recovered standards were calculated as the ratio of the quantity present in the extract and the initial test solution. The concentration ($\mu g k g^{-1}$) of the X compound in the strawberry coulis is calculated with the following expression:

$$C_{\rm X} = C_{\rm E}.K_{\rm X}.\frac{S_{\rm X}}{S_{\rm E}}$$

 $C_{\rm E}$ = concentration of internal standard (µg/kg of fruit),

- $K_{\rm X}$ = response coefficient chromatographic of X compounds as compared to internal standard (for this study, response factor was not determined; we used $K_{\rm X} = 1$),
- $S_{\rm X}$ = peak area of X compound in the organic extract of strawberry,
- $S_{\rm E}$ = peak area of internal standard in the organic extract of strawberry.

2.12. Statistical analysis

Significant variations in the data were identified and analysed using variance analysis (ANOVA) and Student's *t*-test (Sado & Sado, 1991). The differences were considered significant with p < 0.05.

3. Results

The chromatograms that result from pressures of 200, 500, 800 MPa, and the sterilisation process, are shown in Figs. 1–5. Each treatment and analysis was carried out in triplicate. From these chromatograms, 46 compounds have been isolated and identified with the aid of the standard library of compound programmes. They are presented in Table 2.

From the particular pattern of the aromatic profile and by utilising the reference library of chromatograms, it has been possible to identify the particular volatile constituents corresponding to each peak and the odours with which they are associated (Gunther-Douillard, 1988; Hirvi, 1983).



Fig. 1. Gas chromatogram of a fresh strawberry (Gariguette) coulis.



Fig. 2. Gas chromatogram of a strawberry (Gariguette) coulis after high-pressure treatment (200 MPa, 20 min, 20°C).

Once the various compounds that are present had been identified, an attempt could be made at calculating their total mass with respect to each of the sample types (Table 3). This table also summarises whether the difference between the total mass of volatile compounds for each sample type and the untreated reference sample was statistically significant or not. Total volatiles in sterilised samples were significantly higher.

Aroma does not necessarily depend on the quantitative concentration of any volatile compound. Compounds that are highly significant as regards their concentration may have no significance as regards the aroma and, conversely, some compounds that are only present in trace amounts could be very important to the aroma (Gunther-Douillard, 1988). The work of numerous authors (Gunther-Douillard, 1988; Kallio, 1976; Hirvi, 1983; Schreier, 1980) on the aroma of the strawberry, and in particular on the aroma of the cultivar 'Garriguette', has resulted in the discovery of 24 aromatic compounds that play a key role in the aroma (see Table 4). This table also shows whether the values for concentration are significantly different from those of the untreated reference sample. Each aromatic compound was referenced with its retention time but also



Fig. 3. Gas chromatogram of a strawberry (Gariguette) coulis after high-pressure treatment (500 MPa, 20 min, 20°C).



Fig. 4. Gas chromatogram of a strawberry (Gariguette) coulis after high-pressure treatment (800 MPa, 20 min, 20°C).

with the retention index using Kovats methodology. The retention index of the internal standard was measured at 1300.

4. Discussion

The discussion has been arranged by sections. In the first instance, comments are presented on the different aromatic profiles associated with the various treatments compared to the raw coulis reference sample. Discussion then extends to the significance of differences in the concentration of the aromatic compounds, though it must be remembered that no simple relationship exists between the concentration of the aromatic compounds, either singly or in total, and the appreciation of the aroma by the human senses (Butz et al., 1994; Gunther–Douillard, 1988).

4.1. Effect of treatments on aromatic profiles of strawberry coulis

Qualitatively, aroma of Gariguette strawberry is correlated with two main aromatic compounds which are nerolidol (3,7,11-trimethyl 1,6,10 dodecatrien-3-ol) and furaneol (2,5-dimethyl-4-hydroxy-furan-3-one) (Douillard & Guichard, 1989).

No differences between profiles are discernible in comparing the untreated raw coulis with either of the two pressure treatments at 200 and 500 MPa for 20 min



Fig. 5. Gas chromatogram of a strawberry (Gariguette) coulis after sterilization (0.1 Mpa, 20 min, 120°C).

at 20°C (Figs. 1–3). Only concentrations of compounds change slightly (Table 4) but there are no new creations or disappearances from the reference profile. The result for 800 MPa at 20°C for 20 min pressure treatment does, however, show significant differences (Fig. 4). Although no compounds disappear and the relative importance might change, the significant fact is that certain new compounds appear. These new compounds are most probably 3,4-dimethoxy 2-methyl furan or 2,5dimethyl 4-methoxy furan-3 one-3 with a retention time of 12.8 min (Fig. 6) and a γ -lactone with a retention time of 24.3 min (Fig. 7).

The concentration of these two new peaks is 323.12 and 1108.61 μ g/kg, respectively. These new components, are not at all, negligible, above all in the case of the lactone that accounts for approximately 5% of the total of all volatile compounds (Table 3). No explanation can be put forward to explain, with certainty, the presence of these new compounds. At 800 MPa, the temperature inside the vessel rose (12°C) and energy might be sufficient to activate chemical reactions such as condensation or oxidation. It would be very interesting to isolate these new compounds and find out their specific odour through sniffing in order to appreciate whether their role could be considered negative or positive.

In the case of the sterilised sample (120°C, 20 min), many new volatile compounds (significant to a greater or less degree) were found when compared to the profiles of the raw sample and the pressure-treated samples. Substances were identified by retention time. With a retention time of 18.71 min there are low levels of geraniol present (98.8 μ g kg⁻¹), at 21.65 min an unidentified compound is observed (referred to as unknown 'cooked') which is specific to heated foodstuffs and is present in very great quantities, in the order of 3704. µg kg⁻¹. Finally, at 31.81 min vanillin is noticed at a concentration of 83.1 µg kg⁻¹ (Table 4). All these compounds are typical of heated products and it is to be noted that none of them are to be found in the samples that were pressure-treated, or in such small quantities that they could not be taken account of in the analysis that was undertaken (Table 4). It is also pertinent to see how the derivatives of the materials used for packaging the samples appear in the chromatographic profile. In the case of the sterilised coulis, 4 different constituents are noticed. A retention time of 19.90 min is associated

Table 3

Total mass of volatile compounds present in the strawberry (Gariguette) coulis

	Untreated sample	High pressure (200 MPa, 20 min)	High pressure (500 Mpa, 20 min)	High pressure (800 MPa, 20 min)	Sterilisation (120°C, 20 min)
Total mass of volatile	19.2	18.8 NS ^a	19.6 NS	18.9 NS	26.7** ^b
compounds (mg/kg)	± 1.94	±2.69	± 1.67	± 0.77	± 2.92

^a NS, not significant at p < 0.05.

^b **Significant at p < 0.05.

Table 4 Concentration (μ g/kg) of the typical compounds in the aroma of strawberry Gariguette

Ret. time (min)	Retention index (k) ^a	Volatile compounds	Untreated	200 Mpa, 20 min	500 MPa, 20 min	800 MPa, 20 min	Sterilised 120°C, 20 min
2.14	1031	Ethyl butanoate	340 (26) ^b	222*** (25)	350 NS ^c (16)	234***(10)	334NS (37)
2.47	1068	Butyl acetate	103 (7)	121* (10)	112 NS (6)	78*** (3)	175*** (22)
2.56	1079	Hexanal	257 (21)	319* (41)	268* (11)	217*** (21)	583*** (68)
3.88	1176	Heptan-2 one	7 (3)	(4) NS (1)	(4) NS (1)	11** (0.2)	15** (2)
3.98	1183	Methyl hexanoate	839 (69)	624** (77)	840 NS (16)	689** (45)	775 NS (64)
4.49	1213	Hexen-2-al	348 (33)	498** (73)	328 NS (5)	190*** (7)	1101*** (148)
4.78	1229	Ethyl hexanoate	185 (60)	88* (25)	194 NS (19)	95* (4)	69** (5)
5.54	1268	Hexyl acetate	109 (10)	103 NS (11)	101 NS (5)	258*** (12)	117 NS (0.1)
7.31	1349	Hexanol	478 (41)	148*** (16)	450 NS (0.1)	653*** (44)	265*** (26)
8.13	1386	Nonanal	19 (3)	28 NS (9)	20 NS (5)	26 NS (9)	77 NS (77)
10.12	1460	Furfural	40 (8)	44 NS (4)	42 NS (0.1)	62 NS (27)	56 NS (25)
11.91	1543	Linalool	291 (23)	257 NS (28)	303 NS (11)	160*** (1)	671*** (94)
12.26	1560	2-Methyl propanoic acid	84 (9)	83 NS (16)	77 NS (7)	54*** (3)	172*** (6)
13.66	1620	Butanoic acid	149 (17)	174 NS (36)	I56 NS (22)	145 NS (8)	298*** (47)
14.58	1660	Sec-butyric acid	637 (72)	569 NS (108)	61 NS (73)	237*** (24)	1018** (153)
18.60	1830	Hexanoic acid	5099 (568)	4923 NS (894)	5111 NS (591)	4960 NS (53)	9172*** (919)
18.71	1845	Geraniol	_	-	-	-	99 (13)
19.20	1867	Benzene methanol	58 (7)	57 NS (8)	54 NS (4)	136*** (1)	115*** (19)
21.65	1987	Unknown (heat)	_	-	-	-	3704 (345)
22.40	2023	Furaneol	170 (22)	188 NS (26)	168 NS (18)	98*** (1)	509*** (7)
22.59	2034	Nerolidol	243 (24)	245 NS (9)	296** (15)	192* (25)	401*** (22)
22.91	2051	Octanoic acid	148 (11)	128* (9)	162 NS (20)	189*** (8)	257*** (23)
28.53	2125	γ -Decalactone	270 (43)	245 NS (27)	33 NS (69)	141*** (23)	393** (34)
31.81	2551	Vanillin		_			83 (18)
36.39	2801	Cinnamic acid	113 (31)	80 NS (9)	90 NS (12)	582*** (85)	161 NS (40)

^a Kovats index.

^b Standard deviation of the concentration in parentheses.

^c Not significant at p < 0.05.

* *, **, *** Significant at p < 0.1, p < 0.05, p < 0.01 respectively.



Fig. 6. Illustration of the formation of a volatile compound appearing after a high pressure treatment (800 MPa, 20 min, 20°C). The peak is characterised by a retention time of 12.8 min and is attributed to 3.4-dimethoxy 2-methyl furan or 2-5-dimethyl 4-methoxy furan-3 one.

with butylated hydroxytoluene (BHT), an anti-oxidant, at a concentration of 105 μ g kg⁻¹; at 27.75 min, phenol is observed at a concentration of 724 μ g kg⁻¹; at 33.89 min phthalate is found, at a concentration of 163 μ g kg⁻¹ and finally, at 37.07 min, traces of volatile hydrocarbons at a concentration of 121 μ g kg⁻¹. Conversely, the pressure-



Fig. 7. Illustration of the formation of a volatile compound appearing after a high pressure treatment (800 MPa, 20 min, 20°C). The peak is characterised by a retention time of 24.3 min and is attributed to a γ -lactone volatile compound.

treated coulis samples only show traces of the volatile hydrocarbons. A pressure process of 500 MPa is associated with a concentration of the hydrocarbons of 70.8 μ g kg⁻¹, while for 800 MPa it is 53.7 μ g kg⁻¹ (all with common process time of 20 min and temperature of 20°C).

These results bring out an additional benefit of the high-pressure process; not only is the aromatic profile better-preserved in comparison with sterilisation, but there is far less contamination with the plastic derivatives of the packaging compared to sterilisation.

4.2. Effect of treatments on aromatic volatile compound concentrations of strawberry coulis

The average value of the concentration of total volatile compounds in strawberry is 23 mg/kg. For Gariguette, this value is between 27.4 mg/kg and 44.9 mg/kg (Gunther-Douillard, 1988). In our study, this concentration for the raw strawberry product is 19.2 mg/kg. This difference, compared to that observed by Gunther-Douillard, (1988), is due to the fact that samples were frozen before analysis. Kallio, (1976), has noted a decrease of total concentration of aromatic compounds in strawberry as a function of freezing time.

Table 3 shows that high pressure treatments have no significant effect on the global concentration of aromatic compounds. Sumitani, Suekane, Nakatani, and Tatsuka, (1994), have reported the same results regarding peach aroma after a treatment at 400 MPa for 10 min. In the same way, Butz et al., (1994) showed that the global concentration of aromatic compounds from onions did not change after a pressurisation at 300 MPa for 30 min at 25° C.

In case of sterilised samples (120°C, 20 min), the global concentration of aromatic compounds was significantly modified (Table 3). After such a treatment, its value was 26.37 mg/kg while it was only 19.2 mg/kg for raw product. This increase is the consequence of the appearance of new substances due to the thermal treatment, such as geraniol, vanillin and unknown compounds, which are not present in the raw or high pressure samples.

The volatile compounds are treated by family for convenience. However, two compounds are of special significance because of their importance for aroma. Subjective testing of the 'Gariguette' strawberry aroma has shown that both, nerolidol (3,7,11-trimethyl 1,6,10dodecatrien-3-ol) and furaneol (2,5-dimethyl-4hydroxy-furan-3-one) (Figs. 8 and 9) play a dominant role; indeed Douillard and Guichard (1989) demonstrated that they are positive attributes in fresh strawberry flavour. However, in Table 4, it is possible to see that only treatment at 200 MPa did not change concentration of these two compounds.

A large number of authors have underlined the importance of the family of ester compounds in strawberry aroma. These esters play a dominant role both qualitatively and quantitatively (Pyysalo, Suihko & Honkanen, 1977; Schreier, 1980). The particular compounds that are significant are ethyl butanoate, methyl hexanoate, and hexyl acetate (Gunther-Douillard,



Fig. 8. Identification of nerolidol in strawberry (Gariguette) coulis by mass spectrometry.



Fig. 9. Identification of furaneol in strawberry (Gariguette) coulis by mass spectrometry.

1988). The concentrations (and range) of esters present in fresh strawberries are superior to that in strawberries that have been frozen (Gunther-Douillard; Schreier, 1980). These latter authors have shown that there is a drop of 80% in the concentration of esters after freezing for 24 h. In contrast to this, Hirvi (1983), found that the concentration of ethyl butanoate in frozen strawberries was higher to that in fresh strawberries. The reason for this phenomenon is the oxidation of acids following on the thawing of the strawberries. In this study, the testing of the aroma has been carried out after thawing of the samples. In comparing each sample for the presence of esters it appears that the only sample with no significant variation from the untreated raw coulis is the sample processed at 500 MPa. Pressures treatments carried out at 200 and 800 MPa indicate both positive and negative changes in the concentration of esters (Table 4), whereas the sterilised sample shows no significant variations in ester concentrations.

Previous studies have noted that hydroxy esters are insignificant as regards the strawberry aroma, qualitatively and quantitatively. They are only present in trace amounts (Gunther-Douillard, 1988), and they have not been quantified for the purposes of this study.

Aldehydes which are associated with the smell of foliage, grass, ripe fruit or even apple, in the make-up of the strawberry aroma (Flath, Black, Guadagni, Mac-Fadden & Schultz, 1967), do not seem to be much affected by the pressurisation process. Only the amount of hexanal varies significantly when sterilisation treatment or pressure treatment at 800 MPa are applied. Poretta, Birzi, Ghizzoni and Vicini, (1994) have already noted that the presence of hexanal in tomato juice increases after pressurisation at 500 MPa for 3 min. As another illustration, the presence of hexanal has been observed to increase by 40% in the case of onion high pressure-treated at 300 MPa for 30 min (Butz et al., 1994). In the present study, no similar result has been observed; hexanal concentration increases only from 257 to 268 μ g kg⁻¹ after a high pressure treatment at 500 MPa for 20 min.

Alcohols, in particular linalool and nerolidol, play an important role in the strawberry aroma. They bring the scent of flowers (Gunther-Douillard, 1988). No significant difference in the concentration of linalool between fresh and frozen fruit was found by Schreier (1980) or Hirvi (1983). The present study has detected important variations in linalool after pressure treatment at 800 MPa and, for nerolidol, after treatment at 500 MPa (and above). It is important to underline the fact that nerolidol is the only compound that is present in larger quantities in frozen strawberries than fresh fruit (Schreier).

The ranking of strawberries by the strength of their aroma depends very much on the concentration of furaneol (Gunther-Douillard, 1988). This study has shown that its concentration changes little for pressure treatments at 200 and 500 MPa, whereas there is a significant drop from 170 to 98.2 μ g kg⁻¹ for a pressure-treatment of 800 MPa. Hence the change in furaneol can only be considered significant for treatment at 800 MPa.

Acids, such as 2-methylpropanoic, butanoic, hexanoic and octanoic, are predominant in strawberry aroma (Hirvi, 1983; Pyysalo et al., 1977). They have a significant role in the sense that they are precursors in the formation of esters, alcohols and aldehydes (Paillard, 1979). Only heat-sterilisation and pressure treatment at 800 MPa induce significant changes in concentration of organic acid. Among all the acids, only 2-methyl propanoic acid seems to have a positive effect on strawberry aroma (Gunther-Douillard, 1988). In general, acids are associated with unpleasant smells of sweat, rancid butter or stale cheese, except for 2-methyl propanoic which has a slight odour of strawberry at low concentrations (Pyysalo et al.,).

Lactones also play an important role in strawberry aroma, in particular the γ -decalactone (Gunther-Douil-

lard, 1988). The characteristic smell of lactones has been correlated to peaches (Ho et al., 1990) or to fresh coconut (Table 2). The concentration of this lactone is not affected by pressure treatments at 200 or 500 MPa, whereas the modifications are significant for a pressuretreatment of 800 MPa or a heat-sterilisation (Table 4). Similar observations have been made by Sumitani et al. (1994) as regards peach aroma. They found that a sterilisation-treatment (100°C for 30 min) led to a pronounced reduction of the concentration of γ decalactone when compared with a pressure-treatment (400 MPa at 20°C for 10 min). The resulting observed values were 79.1 and 125 g kg⁻¹ for pressure- and thermal-treatment, respectively (Sumitani et al.)

Ketones (3-pentene-2-one-2, heptan-2-one), with their intense fruit and flower flavours, probably have an influence on strawberry aroma, even though it might still be difficult to define. As regards the heptan-2 one in particular, high pressure treatments at 200 and 500 MPa have no apparent effect contrary to that observed at 800 MPa and heat-sterilisation.

Monoterpene (limonene or α -pinene) is characterised by a weak hint of lemon. No trace of this was found, probably because of the choice of FFAP column and the scaling of GCP. Nevertheless, it would not appear to play an important role as a constituent in the aroma of strawberry (Gunther-Douillard, 1988).

5. Conclusion

When a coulis of strawberry (Gariguette) is submitted to a pressure-treatment at 200 or 500 MPa for 20 min, no major alteration in its aromatic profile is observed compared to unprocessed fruit. A comparison of the respective chromatograms shows no deletions or additions of peaks compared to the reference sample. Furthermore, there is no significant change in the relative concentrations of the various compounds compared to the unprocessed sample.

The aromatic profile resulting from the strawberry coulis, treated at 800 MPa, is very different, showing significant differences with the untreated and the 200 and 500 MPa results. New compounds are detected (3,4-dimethoxy 2-methyl furan and (γ -lactone) and the relative importance of the remaining substances changes significantly with respect to the untreated sample.

Comparisons with heat sterilised samples are quite striking. Typical volatile compounds due to heat sterilisation, such as geraniol, vanillin, or an unidentified compound are not present with pressure-treatment. Global concentration of strawberry volatiles increases in the sterilised coulis due to the creation of new compounds and to the increase of concentration of typical strawberry compounds, such as hexen-2-al, linalool or hexanoic acid, probably favoured via energy developed by the sterilisation. Furthermore, the presence of derivatives from the packaging is far more significant in the case of heat-sterilisation than in the case of the pressure treatments, even at 800 MPa.

These results refer to analytical investigations and not to sensory tests. Thus, any interpretation relative to their influence of sensory profile is not sufficient. It would be interesting to correlate analytical data with sensory analysis.

Furthermore, it would be desirable to examine the characteristics of the specific samples through time to see what changes, if any, occur, because all the samples in this study were tested only a short time after treatment.

In conclusion, high-pressure treatment would appear to present several advantages for the preservation of highly aromatic foodstuffs. In fact, it provides a good protection from microbiological contamination (Aleman et al., 1996; Butz & Tauscher, 1997; Cheftel, 1995; Donsi, Ferrari & Di Matteo, 1996; Poretta et al., 1995) while, at the same time, most of the aromatic constituents of the products, at least at the pressures usually developed in industry (between 200 and 500 MPa), are preserved. This is not specific to strawberries because Sumitani et al. (1990), have recommended high-pressure treatment for pieces of peach in order to conserve their aromatic qualities.

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