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Optimization of Extraction of Apple Aroma by Dynamic Headspace and Influence of Saliva on Extraction of Volatiles

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The dynamic headspace procedure of aroma extraction was optimized on Gala apples (*Malus domestica*). Two parameters affecting the extractability of compounds were studied: temperature and purge time. The influence of artificial saliva was also included. An increase in purge time and temperature caused an increase in the extraction of volatiles from the apple matrix. The optimum point of extraction was 40 °C and 70 min of purge. The study also showed that the addition of saliva influenced the extraction of volatile compounds, but this effect was different from one compound to another. To verify that the headspace extracts presented a global odor representativeness of fresh apple under these conditions of extraction, eight assessors compared the odor of extracts with fresh fruit odor for three different cultivars. With regard to the sensory profiles of extracts, the optimal conditions of extraction were suitable for extraction of volatile compounds, even if cooked apple odor appeared in some extracts. The similarity marks of extracts were low but acceptable.

KEYWORDS: Apple; aroma; dynamic headspace; optimization; artificial saliva; odor representativeness

INTRODUCTION

The volatile constituents of apples have been studied for over 50 years, and over 300 volatile compounds have been found in different apple cultivars. Only a few of these volatiles have been identified as being responsible for apple aroma (*1*). The most abundant volatile components in apples are esters (78−92% of total volatiles), alcohols (6−16% of total volatiles), aldehydes, ketones, and ethers (*1*, *2*).

In recent studies, dynamic headspace gas chromatography is the most commonly used technique for apple aroma analysis (*3*, *4*). It has been advantageous for several reasons: (1) qualitative and quantitative changes that can accompany other sample preparation procedures are eliminated (e.g., no solvent elution) (*5*, *6*); (2) the introduction of nonvolatile residues into the column is avoided (*6*); and (3) it is a gentle and fast workup procedure (*5*, *6*).

The disadvantage of this technique is that it enables only the low boiling point components to be extracted (*7*). Therefore, we decided to optimize this method on apples for parameters that should increase the extractability of components. Among these parameters, we can cite temperature and purge time.

During the past 10 years, many studies have focused on the role of saliva in the extraction of volatile components (*8*−*10*). Artificial saliva is most frequently composed of mineral salts (NaCl, KCl, $CaCl_2$), α-amylase enzyme, and mucins. Konczal et al. (*11*) have studied the influence of NaCl on the extraction of volatile components from apple juice and have shown it to be better due to the effect of "salting out". The addition of salt causes a decrease in hydrogen bonding between the analyte and water as free water is sequestered by the tight hydration shell surrounding the salt ion. However, to date, no work has been published on the influence of saliva on the extraction of volatiles from such a complex matrix as an apple. Thus, we decided to include the influence of artificial saliva in our experimentation. With this objective, two central composite designs (CCD) with two factors (temperature and time) were established: one with artificial saliva and the other without artificial saliva.

Generally, an extraction method is optimized when it is repeatable and discriminatory and gives an aroma extract that is close in sensory terms to the product (representative). Many authors have highlighted the importance of testing the odor representativeness of extracts (*12*−*15*). The odor representativeness can be defined as a similarity between the extract odor and the product odor, and the test of representativeness is based on the sensory evaluation of extract and product by a trained panel. This test should be a prerequisite to further analysis. In our study, the experimental designs were composed of 22 essays (2 CCD × 11 essays), which means that we should test the representativeness of 116 extracts (22 × 8 assessors). This experimentation would be too long, and the fruits would change between the first and the last essay. Thus, the optimization of the headspace method was not performed in relation to the odor representativeness of the extracts. Instead, we chose to study, in response to CCD, the total quantity of odorant volatiles (TQOV) known in the bibliography (*1*) to contribute to apple aroma ("odor active" compounds). At the same time, we studied the total quantity of extracted volatiles (TQV) in order to

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compare their behavior to that of TQOV. Nevertheless, when the optimization was finished, the test for odor representativeness was carried out on extracts obtained in optimized conditions with three different apple cultivars. To our knowledge, there are no studies of odor representativeness of apple extracts obtained with the dynamic headspace method. The odor of each extract was compared to the odor of fresh apple fruit.

The main objective of the study was to determine the effects of temperature and extraction time on composition of apple extracts obtained by dynamic headspace method and to find the optimum extraction conditions for this extraction method. In addition, we aimed to study the influence of saliva on the extraction of volatiles from such a complex matrix as an apple. Then, the test of odor representativeness of extracts obtained in optimized conditions was performed to verify that the headspace extracts presented a global odor representative of fresh apple fruit in these conditions of extraction.

MATERIALS AND METHODS

Apples. The optimization of the dynamic headspace method of extraction was done with Gala apples (*Malus domestica*). The test for odor representativeness, which was done afterward, was not done on Gala apples. We chose to carry out this test on three apple cultivars that represent a wide range of aromas and flavors and have very different sensory characteristics. These cultivars were Golden Delicious, Braeburn, and Fuji.

All apples were harvested in 2002, at commercial maturity, as determined by different tests (diameter, firmness, and color), from Pays de Loire, France. Immediately after picking, fruits were selected for their uniformity (diameter, absence of damage or blemishes, ground color) and stored at 3 °C for 3 weeks in the same cool room. They were kept at ambient temperature for 24 h before extractions.

Reagents. Water was purified by a Milli-Q system (Millipore Corp., Molsheim, France). *n*-dodecane (99.9%), mucin, porcine α-amylase, and NaCl (99.5%) were purchased from Sigma-Aldrich Chemical Co (Saint-Quentin-Fallavier, France). Other salts, $NaHCO_3$ (99.5%), NaN_3 (99.5%), KCl (99.5%), K_2HPO_4 (98%), and $CaCl_2 \cdot 2H_2O$ (97%), were purchased from Merck (Fontenay-sous-Bois, France) The Teflon bags came from Interchim (Sacs de prélèvement ref.: 322600, 300 mL, Montluçon, France).

Artificial saliva (*16*) was prepared by dissolving in 250 mL of water $NaHCO_3$ (1.3020 g), NaCl (0.2193 g), NaN_3 (0.1250 g), KCl (0.1193 g), $CaCl_2 \cdot 2H_2O$ (0.1102 g), K_2HPO_4 (0.2612 g), mucin (0.540 g), and porcine α-amylase (3.2504 g or 50000 units) (pH adjusted to 7).

Experimental Design. A CCD consists of a complete $\mathbf{2^k}$ factorial design, where the factor levels are coded by −1 and +1 values, n_0 center points, and two axial points on the axes of each design variable at a distance α from the design center.

In our case, two CCDs were established: with and without saliva. Each CCD contained two factors: temperature and time ($\mathbf{k} = 2$). The central point was repeated three times ($n_0 = 3$), and there were four axial points, which means that each CCD was composed of 11 randomized experimental points. All experiments were finished in one week so that there was not much difference between the apples used in the first and in the last assay.

In previous studies, the temperature of extraction of apple aroma by headspace methods varied between ambient temperature and 40 °C, whereas time varied between 30 min and 2 h (*3*, *17*, *18*). That is why, for our experimental design, the limits fixed for temperature were 25 °C (ambient temperature) and 40 °C (maximal temperature used in the literature for extraction of apple volatiles) and the limits for time were 30 and 70 min.

Dynamic Headspace Extraction. For each assay, four randomly selected fruits were cut in half and their cores discarded. Two equal longitudinal slices (3 mm thick) were cut from each half-apple. Then each slice was cut again into eight. Twenty grams of the sample was introduced into a flask (50 mL) containing stir bars. The sample was covered by either water (30 mL) or a mixture of water and artificial saliva (26 and 4 mL, respectively). Next, *n*-dodecane was introduced (20 μg) into the mixture as internal standard. This compound was chosen because its extraction by headspace was repeatable (yield of 65%) and also because its retention index on a capillary column was different from that of the other compounds extracted from apples. As soon as the internal standard was introduced, the flask was hermetically closed and connected to a purge and trap concentrator (model 3000; Tekmar Inc., Cincinnati, OH) equipped with a capillary interface for cryofocusing. The temperature during extraction is maintained constant with a water bath. To avoid the generation of oxidation artifacts, a 2 min prepurge was included to remove air from above the sample. The sample headspace was then purged with helium at 60 mL min^{-1}. The time and temperature were dependent on each assay. The volatiles were swept into a porous adsorbent polymer (Tenax 1.8 in. × 12 in.) trap, which was maintained at 30 °C. Then they were cryofocused at −40 °C using carbon dioxide and thermally desorbed at 195 °C. During the desorption, volatiles were transferred directly into the column through the injection port in splitless mode.

Qualitative and Quantitative Analysis of Extracts. For the identification and confirmation of compounds, a Perkin-Elmer mass spectrometer coupled to a gas chromatograph (GC-MS) was used. Volatile compounds were separated on a capillary column (DB-Wax, 30 m in length × 0.25 mm i.d. × 0.5 μm thickness, J&W Scientific, Folsom, CA). The helium carrier gas linear velocity was 32 cm s^{-1}. The injector and detector were at 250 °C. The oven temperature was programmed from 40 °C for 10 min at 2 °C min^{-1} to 100 °C, followed by a temperature increase of 15 °C min^{-1} to 230 °C (15 min). MSD (electronic impact ionization) conditions were as follows: ionization energy, 70 eV; mass range, 33−300 amu; scan rate, 2.0 scan s^{-1}; electron multiplier voltage, 2000 V.

The volatile compounds were identified by matching their spectra to those in the NIST and Wiley NBS mass spectra library. The retention index of each volatile compound, calculated according to the method of ref *19*), was compared with those in the literature. Chemical standards of some volatile compounds were directly injected into the GC-MS.

For quantitative analysis, a gas chromatograph (Star 3400 Cx, Varian, Palo Alto, CA) equipped with a flame ionization detector was used. The volatile compounds were separated on a capillary column (DB-Wax, 30 m in length × 0.32 mm i.d. × 0.5 μm thickness, J&W Scientific). The helium carrier gas linear velocity was 53 cm s^{-1}. The injector and detector were set at 250 °C. The oven temperature was programmed from 40 °C for 10 min to 100 °C at 2 °C min^{-1}, followed by a temperature increase of 15 °C min^{-1} to 230 °C (15 min). Quantitative results are expressed in quantity equivalents of *n*-dodecane per kilogram of fruit.

Sensory Analysis. *Panel.* Eight assessors were recruited in our laboratory and trained in aroma recognition with the "Field of Odors" by Jaubert et al. (*20*). Then they were all trained on apple aroma recognition (eight 1-h sessions) as described previously by Mehinagic et al. (*21*). The first session took place in an ordinary room in order to generate descriptors for the cultivars Golden Delicious, Braeburn, and Fuji, which represent a wide range of aromas and tastes. The other sessions were held in a sensory room (*22*), in isolated booths, under red light at ambient temperature. The panel generated descriptors for fresh apples. A list of 14 consensual descriptors was established: banana, bluegrass, caramel, cinnamon, honey, cooked apple, cucumber, fermented, fruity, grass, pear, strawberry, sweet, and woody. The final descriptors and their corresponding standards were chosen by the assessors after discussions during training. Chemical standards were diluted in ethanol (40 μL mL^{-1}), and the determined quantity for each diluted standard was put on the odor blotter strips in brown flasks (**Table 1**).

Preparation of the Sample. Volatile extracts were collected at the end of the dynamic headspace interface with an original technique developed in our laboratory (*23*). The extraction was done in optimal conditions of time and temperature with no addition of internal standard. The volatiles were swept into a porous adsorbent polymer (Tenax) trap. Then they were cryofocused at −40 °C using carbon dioxide and thermally desorbed at 195 °C for 2 min. During the desorption, volatiles were transferred directly into a piece of deactivated silica column. The helium flow through it was 60 mL min^{-1}. This piece of deactivated

Table 1. Attributed Descriptors and Reference Standards[a] for Descriptive Sensory Analysis of Fresh Apple Fruit and Its Extracts

descriptor	reference standard
banana	banana
bluegrass	bluegrass
caramel	ethyl maltol (30 μL of diluted standard)[b]
cooked apple	apple pie
cinnamon	cinnamic aldehyde (120 μL of diluted standard)
cucumber	cucumber
fermented	baker's yeast
fruity	ethyl butyrate (30 μL of diluted standard)
grass	*Z*-3-hexen-1-ol (30 μL of diluted standard)
honey	honey
pear	Pear Williams
strawberry	strawberry
sweets	isoamyl acetate (60 μL of diluted standard)
woody	α-santalol (120 μL of diluted standard)

[a] All chemical standards were purchased from Sigma-Aldrich Chemical Co. (Saint-Quentin-Fallavier, France) except ethyl butanoate, which was purchased from VWR International (Fontenay-sous-Bois, France). [b] Diluted standards were prepared by diluting the chemical standards in ethanol (40 μL mL^{-1}).

column, 30 cm in length, transferred volatile compounds directly into a special Teflon bag (300 mL), designed to be odorless and leakproof, as described by Mehinagic et al. (*21*). To avoid leakage of volatiles from the Teflon bag, the deactivated column was passed through a needle that was fixed on the top of the bag. At the end of the desorption, the needle set was closed with a Teflon cup. The needle set is opened just before the sensory analysis; after an equilibration time of 10 min, the needle set directed the odor extract to a glass nose cone fitted on the Teflon bag. One Teflon bag was prepared for each assessor, and each extract was analyzed immediately after the aroma recovery.

Odor Representativeness Test. Three sessions were organized for each assessor for three different apple cultivars. The sessions were held in a sensory room, in isolated booths, under red light at ambient temperature. An extract was prepared separately for each assessor. Fresh apple fruits were cut in pieces and sealed in round amber glass bottles, just before sensory analysis (during 5 min at room temperature) to limit the oxidation of the fruit. Each assessor had to assess the extract and to compare it to that of the fresh sliced apples. They first evaluated the intensity of the 14 given descriptors for the extract and then for the sliced apples, used as a reference, by sniffing the odors liberated from the bottle. Next, they assessed the similarity of the extract odor compared to that of a fresh apple, as well as the global intensity of the extract odor. A continuous scale of 100 mm was used for evaluation. The left side of the scale corresponded to the lowest intensity (note 0) and the right side corresponded to the highest intensity (note 10).

Statistical Treatment. Data acquisition and statistical treatment were performed with Statgraphics Plus 5.1 software (Sigmaplus, Toulouse, France).

The studied responses were TQOV and TQV.

To analyze the repeatability of the extraction method, we compared triplicates of the central point of the two experimental designs (32.5 °C and 50 min).

A two-way ANOVA, with a 95% confidence level, was then performed to verify if time and temperature had a significant effect on aroma extraction. Possible significant differences between response values were evaluated by least significant differences (LSD) multiple-comparison tests with a confidence level of 95%. Finally, main, interactive, and second-order effects for each response were calculated by multiple linear regression analysis (*24*) and were presented with estimated surface responses.

Sensory data were standardized before principal component analysis (PCA), and the relationship between sensory descriptors was studied.

RESULTS AND DISCUSSION

Qualitative Results and Repeatability of the Extraction Method. Twenty volatile compounds were identified in all headspace extracts (**Table 2**) and included esters (14 compounds), alcohols (4 compounds), and aldehydes (2 compounds). All of these compounds have already been identified in apples. A common feature of all the extracts obtained is the predominance of esters. The most abundant esters are acetic ester types (7 esters), then butanoate ester types (4 esters), propanoate ester types (2 esters), and hexanoate ester types (1 ester). Alcohols and aldehydes are less abundant in apples at this ripening stage, but some of them contribute to apple aroma (*25*).

Table 2. Compounds Identified from Gala Apple

TQV[a]	TQOV[b]	identification[c]	odor	threshold, mg L^{-1} (*ref*)
ethyl acetate		MS, RI, std	fruity	5 (*35*)
ethanol		MS, RI, std	alcohol	1000 (*35*)
propyl acetate		MS, RI, std	pleasant, mild, fruity	2 (*1*)
2-methylpropyl acetate		MS, RI	fruity, pear	0.065 (*36*)
ethyl butanoate	X	MS, RI	fruity, estery	0.001 (*35*)
ethyl 2-methylbutanoate	X	MS, RI, std	fruity	0.000006 (*36*)
butyl acetate	X	MS, RI, std	red apple, nail polish	0.066 (*35*)
hexanal	X	MS, RI, std	green, green apple	0.005 (*35*)
2-methylpropanol		MS, RI	sweet, musty	5.3 (*37*)
2-methylbutyl acetate	X	MS, RI, std	apple	0.011 (*36*)
Z-3-hexenal	X	MS, RI, std	green apple	0.00025 (*38*)
butyl propanoate	X	MS, RI, std	fruity, apple	0.025 (*36*)
butan-1-ol	X	MS, RI, std	sweet	0.5 (*39*)
pentyl acetate	X	MS, RI, std	banana, apple, fruity	0.005 (*39*)
butyl butanoate	X	MS, RI, std	rotten apple, cheesy	0.1 (*36*)
butyl-2-methylbutanoate	X	MS, RI	apple, fruity	0.017 (*36*)
hexyl acetate	X	MS, RI, std	red apple, pear	0.002 (*35*)
hexyl propanoate	X	MS, RI, std	apple, fruity	0.008 (*36*)
hexan-1-ol	X	MS, RI, std	earthy	0.150 (*37*)
butyl hexanoate	X	MS, RI, std	green apple	0.7 (*36*)

[a] Total quantity of volatiles. [b] Total quantity of odorant volatiles identified in the literature as odor active compounds. [c] MS, compound was identified by matching its spectra to those of the NIST and Wiley NBS mass spectra library; RI, compound was identified by chromatographic retention index on a DB-WAX column; std, compound was identified by chromatographic mass spectrometric comparison with an authentic standard.

We identified in the extracts 15 compounds known to be "odor active" in the literature (*4*, *26*): ethyl butanoate, ethyl 2-methylbutanoate, butyl acetate, hexanal, 2-methylbutyl acetate, *Z*-3-hexenal, butyl propanoate, butan-1-ol, pentyl acetate, butyl butanoate, butyl 2-methylbutanoate, hexyl acetate, hexyl propanoate, hexan-1-ol, and butyl hexanoate. All of these compounds have low threshold values (**Table 2**).

To verify the repeatability of the extraction method, a one-way ANOVA, with a 95% confidence level, was performed on results obtained from the repetition of the central points from CCDs with and without saliva. As we were particularly interested in 15 odor active compounds, 90 results (15 responses $\times$ 3 repetitions $\times$ 2 CCD) were considered in order to determine if there were significant differences between responses.

ANOVA showed that there were no statistically significant differences between replicates of the central point of the two experimental designs with a 95% confidence level (p value = 0.80), so the repeatability of the extraction technique is satisfying.

Influence of Time and Temperature. The results of the experimental designs with TQOV and TQV values are shown in **Table 3**. The highest values for TQOV were obtained at 40 °C and 70 min, whereas the highest values of TQV were obtained at 43.1 °C and 50 min of extraction.

A two-way ANOVA showed that the main effects of temperature and time are statistically significant, with a 95% confidence level, for TQOV and TQV in both designs (with and without saliva) (**Table 4**). The secondary effects were not

Table 3. Results of Two Central Composite Designs (without and with Saliva)

			without saliva		with saliva	
essay	temp (°C)	time (min)	TQV[a] (mg equiv kg^{-1})	TQOV[b] (mg equiv kg^{-1})	TQV (mg equiv kg^{-1})	TQOV (mg equiv kg^{-1})
1	32.5	50	21.41	19.02	19.09	17.49
2	25	70	20.12	9.67	16.49	15.02
3	40	70	27.57	25.20	43.70	34.71
4	32.5	78.3	21.41	18.89	28.66	24.45
5	40	30	12.99	11.68	14.31	12.68
6	32.5	50	19.76	16.63	19.01	17.34
7	21.9	50	13.28	12.12	16.11	14.93
8	25	30	9.39	8.52	10.38	9.08
9	43.1	50	29.34	16.43	47.17	30.01
10	32.5	21.7	13.93	11.37	10.84	9.86
11	32.5	50	21.08	17.19	24.36	21.14

[a] Total quantity of volatiles. [b] Total quantity of odorant volatiles identified as odor active compounds.

Table 4. ANOVA Applied to the Results of the Central Composite Design

	without saliva				with saliva			
effect	mean square	Df	*F*	*p*	mean square	Df	*F*	*p*
TQOV[a]								
temperature	0.077	1	14.29	0.01	0.249	1	51.72	0.01
time	0.080	1	14.93	0.01	0.293	1	60.74	0.01
temperature^{2,b}	0.020	1	3.91	0.10	0.011	1	2.44	0.18
time^{2,b}	0.012	1	2.31	0.19	0.008	1	1.80	0.24
temp × time	0.038	1	7.19	0.05	0.064	1	13.42	0.01
TQV[b]								
temperature	0.142	1	13.34	0.01	0.704	1	38.04	0.01
time	0.160	1	15.06	0.01	0.460	1	24.86	0.01
temperature^{2,b}	0.000	1	0.02	0.88	0.104	1	5.61	0.06
time^{2,b}	0.023	1	0.35	0.58	0.015	1	0.84	0.40
temp × time	0.004	1	2.18	0.20	0.135	1	7.31	0.04

[a] Total quantity of odorant volatiles identified in the literature as odor active compounds. [b] Total quantity of volatiles.

statistically significant, but the interactions between time and temperature are significant, particularly in the design with saliva (**Table 4**).

Figure 1 shows that an increase in temperature and time produces an increase in TQV when saliva is not introduced. The response surface is planar. Moreover, the increase in time produces an increase in TQOV. Temperature seems to have less effect on TQOV at 30 min than at 70 min. Consequently, the surface responses of TQOV and TQV are slightly different. To understand that difference, odorant compounds were observed separately.

The ANOVA showed that, in the design without saliva, only 5 of the 15 odorant compounds seem to be influenced significantly by an increase in time and temperature: butan-1-ol, pentyl acetate, butyl butanoate, hexyl propanoate, and hexan-1-ol. The influence of temperature on volatile compounds depends not only on their molecular properties (molecular weight, dipole moment) but also on their physical properties (boiling points and solubility). These properties can affect the magnitude of intermolecular forces (*27*). In the liquid phase, the intermolecular attraction forces are very high and hold compounds together, whereas in the vapor phase these attraction forces are negligible. If a chemical has high intermolecular attraction, generally resulting from dipole−dipole or charge−dipole interactions, then the rate of evaporation for such molecules is low. The higher the boiling point of a compound,

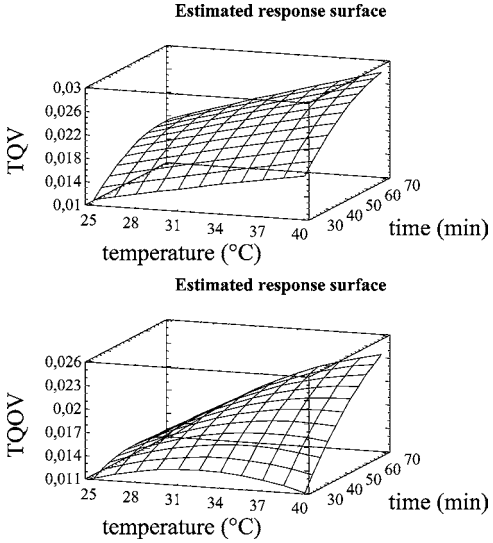

Figure 1. Estimated response surface of different models obtained for total quantity of extracted volatiles (TQV) and total quantity of odorant volatiles (TQOV) in central composite design without saliva.

Table 5. Some Physical Properties of Apple Odor Active Compounds

compound	bp[a] (°C)	solubility[b] (mg/L)	vapor pressure[e] (mmHg)
ethyl butanoate	120[d]	4900 (20 °C)[e]	12.8 (20 °C)[f]
ethyl 2-methylbutanoate	132[d]		
butyl acetate	126[e]	8400 (25 °C)[f]	11.5 (25 °C)[f]
hexanal	130[d]	5640 (30 °C)[f]	11.3 (25 °C)[f]
2-methylbutyl acetate	142[d]	2000 (25 °C)[f]	5.6 (25 °C)[f]
Z-3-hexenal			
butyl propanoate	145[d]	1500 (20 °C)[f]	4.42 (25 °C)[f]
butan-1-ol	118[e]	63200 (25 °C)[f]	6.7 (25 °C)[f]
pentyl acetate	149[e]	1150 (25 °C)[f]	12.1 (25 °C)[f]
butyl butanoate	164[d]	500 (20 °C)[f]	1.81 (25 °C)[f]
butyl 2-methylbutanoate	175[d]		
hexyl acetate	168[d]	511 (25 °C)[f]	1.32 (25 °C)[f]
hexyl propanoate	190[d]		
hexan-1-ol	157[e]	5900 (25 °C)[f]	0.928 (25 °C)[f]
butyl hexanoate	207[e]	33.4 (25 °C)[f]	0.211 (25 °C)[f]

[a] Boiling point at 760 mmHg. [b] Concentration in the water phase when the pressure of the compound above the solution is 101.325 kPa (1 atm). The temperature is mentioned in parentheses. [c] Vapor pressure expressed in mmHg at the temperature mentioned in parentheses and 1 atm. [d] Reference *42*. [e] Reference *43*. [f] Reference *44*.

the more energy must be applied to liberate the chemical to the vapor phase (*27*). Most of the volatile compounds that were influenced significantly by temperature in our study have high boiling points and need more energy, provided by heating, to be liberated (**Table 5**).

Solubility also plays an important role in the extractability of compounds. In general, raising the headspace temperature decreases the partition coefficient (k) of volatiles (*28*). The relationship between ln(k) and reciprocal temperature is linear, but the slope differs from one compound to another. The more soluble the volatile substance, the greater is the change in solubility for a given temperature change (*28*). For example, the boiling point of butan-1-ol is lower than that of hexyl acetate but, because it is polar, it is much more soluble in water than hexyl acetate (**Table 5**). This could explain partially the influence of temperature on the extraction of butan-1-ol. Hexyl acetate is influenced only by purge time and not by temperature, and all other compounds are not significantly influenced by changes in temperature.

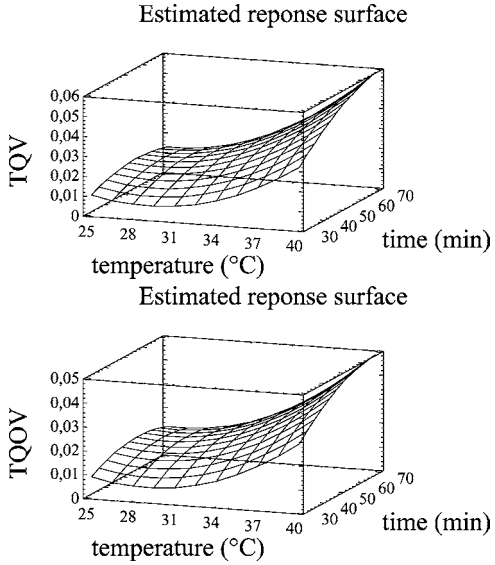

Figure 2. Estimated response surface of different models obtained for total quantity of extracted volatiles (TQV) and total quantity of odorant volatiles (TQOV) in central composite design with saliva.

Table 6. Quantity of Compounds (Milligram Equivalents of *n*-Dodecane per Kilogram of Fruit) Extracted by Dynamic Headspace at Optimal Conditions of Extraction (40 °C and 70 min)

volatile compound	without saliva	with saliva	% change with saliva
TQV[b]	27.57	43.69	58
TQOV[a]	25.2	34.63	37
ethyl butanoate	0.01	0.06	485
ethyl 2-methylbutanoate	0	0.05	
butyl acetate	6.93	15.36	122
hexanal	0	1.82	
2-methylbutyl acetate	2.50	1.28	–49
Z-3-hexenal	0.01	0.02	100
butyl propanoate	0	0.14	
butan-1-ol	6.14	9.05	47
pentyl acetate	0.38	0.50	30
butyl butyrate	1.35	2.19	63
butyl 2-methylbutanoate	0.07	0.17	143
hexyl acetate	4.54	3.64	–20
hexyl propanoate	2.46	0	
hexan-1-ol	0.79	0.35	–55
butyl hexanoate	0.00	0.00	

[a] Total quantity of odorant volatiles identified in the literature as odor active compounds. [b] Total quantity of volatiles.

Figure 2 shows that TQOV and TQV surface responses are both influenced by temperature and purge time when saliva is added. This figure also reveals that only a temperature >32 °C produces an increase in volatiles. The influence of saliva will be discussed below. As the highest amounts of TQOV were observed at 40 °C and 70 min, these conditions will be considered as optimal conditions.

Influence of Saliva. Table 6 shows the influence of saliva on the extraction of 15 odor active compounds at optimal conditions (40 °C and 70 min). Most of the compounds were positively influenced by the addition of saliva: ethyl butanoate, ethyl 2-methyl butanoate, butyl acetate, hexanal, *Z*-3-hexenal, butyl propanoate, butan-1-ol, pentyl acetate, butyl butanoate, and butyl 2-methylbutanoate. On the contrary, the addition of saliva decreased the extraction of 2-methylbutyl acetate, hexyl acetate, hexyl propanoate, and hexan-1-ol.

The influence of saliva can be explained by different phenomena, and the first one could be the "salting-out" effect. The increase in headspace concentration with salt addition is presumably related to the reduction in the available solvent in the liquid phase, resulting from the presence of the nonvolatile solute (salt) (*29*, *30*). Poll and Flink (*31*) studied the influence of salt addition on the headspace volatile composition from apple juice. They observed that the degree of headspace enrichment resulting from salt addition is different for esters, aldehydes, and alcohols. In their collection system, the average degree of enrichment at 40 °C (described in terms of relative peak areas) was greater than 4 for alcohols, 3.5 for aldehydes, and 1.75 for esters. The addition of NaCl to apple juice had almost no effect on esters, whereas the headspace concentration of alcohols and aldehydes increased. In our study, the addition of saliva influenced the increase in alcohols (butan-1-ol) and aldehydes (hexanal and *Z*-3-hexenal), but it also influenced the amount of esters (ethyl butanoate, 2-methylpropyl acetate, and butyl 2-methyl butanoate). In fact, contrary to the results of Poll and Flink (*32*), the extraction of the majority of esters (7/10) was very positively influenced by saliva addition, whereas the extraction of hexan-1-ol decreased with the addition of saliva (**Table 6**). This can be explained by the interactions that could be created between some compounds and other constituents of saliva: α-amylase or mucin. Van Ruth et al. (*9*, *16*) showed that 1-octen-3-ol was released in significantly greater amounts from dried bell peppers and French beans when saliva without α-amylase was added in comparison with artificial saliva containing α-amylase. This could be due to the fact that α-amylase might affect aroma release as a protein. Many studies have shown that proteins decrease aroma release (*32*−*34*). However, the principle action of α-amylase is its enzymatic effect. This enzyme can influence aroma release in food products containing starch, and the optimum activity of our enzyme is obtained at 40 °C. The amylose fraction of starch can form helical structures in which the hydroxyl groups are oriented to the outside of the coil. Consequently, hydrophobic regions exist in the inside of the polymer, in which flavor can be retained (*9*). Therefore, when saliva is added to the extraction, α-amylase can hydrolyze the starch from apples and liberate the volatiles that were captured in the starch structure. However, more experimentation should be done to verify this hypothesis, because the influence of the exogenous enzyme application on apple slices is unknown. Van-Ruth et al. (*16*) showed that mucin could also create interactions with different compounds, and this could decrease the extractability of some compounds. This hypothesis should also be explored on the volatiles found in apples.

In summary, the extraction of TQOV was increased with saliva addition. We observed that the degree of headspace enrichment resulting from saliva addition is different for different compounds. No general observation could be established for esters, alcohols, and aldehydes.

Sensory Evaluation. The previous result showed that the most abundant odorant volatiles were obtained at 40 °C with 70 min purge time and with the addition of saliva. To verify if the apple extracts in these conditions of extraction are representative of fresh apple fruit, sensory analysis was carried out on extracts obtained from three different apple cultivars.

Three different extracts were analyzed by eight assessors. They evaluated the intensity of the 14 sensory descriptorss, and then they assessed the similarity of the extract odor compared to a fresh apple, as well as the global intensity of the extract odor. The LSD multiple-comparison tests, which compared any two means at a confidence level of 95%, showed that there were no statistically significant differences between the Braeburn

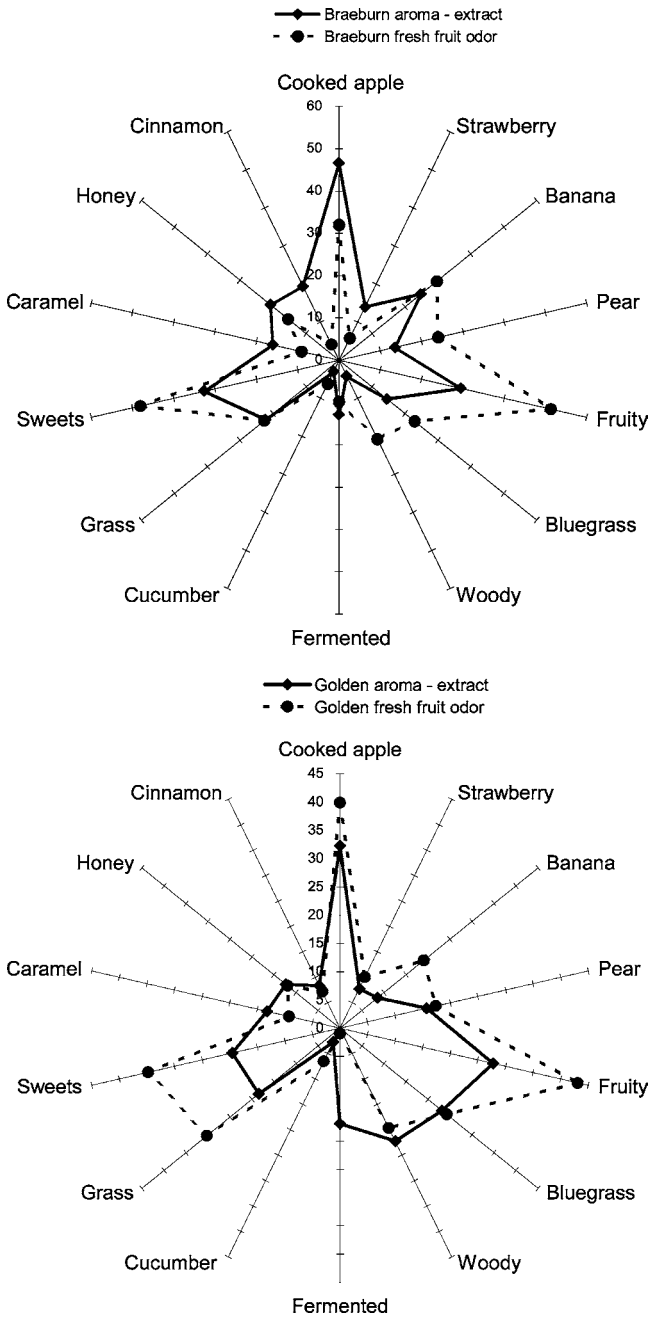

Figure 3. Plots of average sensory scores of Braeburn and Golden Delicious aroma extracts compared to fresh apple fruit.

aroma and fresh Braeburn fruit for any of the 14 sensory descriptors. The same results were obtained for Golden Delicious apples, whereas the extracts obtained with Fuji were statistically different from fresh fruits for cooked apple descriptor. This can be visualized on the plots of averaged sensory scores of the extracts compared to the fresh apple fruits (**Figures 3** and **4**). **Figure 3** shows that sensory profiles of Braeburn and Golden apples are very similar to the profiles of their extracts for all descriptors except for fruity and sweet. **Figure 4** shows that the extracts obtained with Fuji apples are characterized with higher cooked apple odor than the fresh fruit.

Table 7 shows that the intensity marks of extracts were quite high and similar, especially for the varieties Fuji and Braeburn. It also shows that there was no significant difference among the three extracts for the similarity marks. The intensity mark of the Golden Delicious apple extract is lower (51.2 mm) than the intensity marks of the other two extracts, whereas its

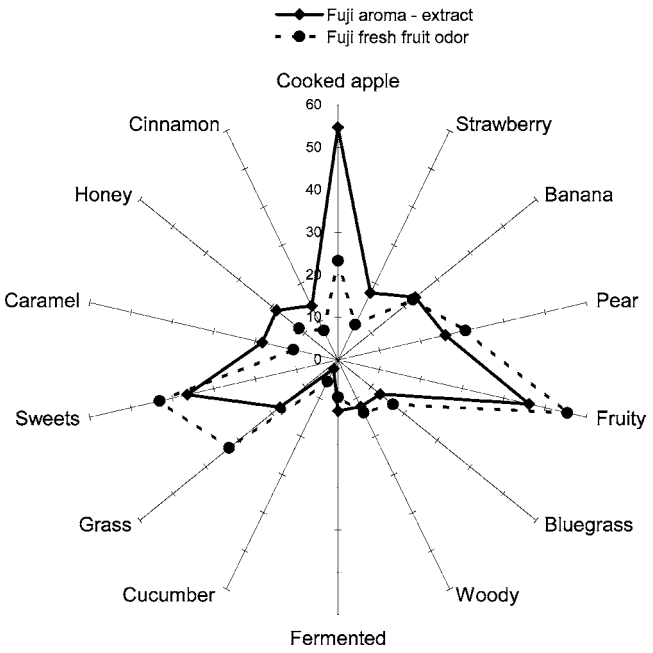

Figure 4. Plot of average sensory scores of Fuji aroma extracts and fresh apple fruit.

Table 7. Odor Intensity Evaluation and Similarity Marks of Extracts of Golden Delicious, Fuji, and Braeburn Apples[a]

extract	intensity marks (100-mm unstructured scale), mm	orthonasal similarity to odor of apples (100-mm unstructured scale), mm
Golden Delicous	51.2 a	50.6 a
Fuji	70.3 b	42.4 a
Braeburn	68 b	49.4 a

[a] This table is based on homogeneous groups for extracts calculated by LSD at 95% confidence level. Entries followed by the same letter were not significantly different.

similarity marks are the highest. The similarity marks for odor were acceptable but not very high (between 42.4 and 50.6 mm on a 100-mm unstructured scale) (**Table 7**). This is in accordance with the results of Escudero and Etievant (*40*), who obtained a mean score of 47.4 mm on a 100-mm unstructured scale for Champagne extracts, and with those of Le Quere et al. (*41*), who obtained a mean score of 44 mm on a 100-mm unstructured scale for cheese extracts. The low similarity marks can be explained not only by differences in the intensity notes of the extracts but also by the "psychological effect". Indeed, the assessors compared the apple aroma extracts in Teflon bags and the odor liberated from the fresh fruit. Le Quere et al. (*41*) have already shown that when assessors evaluated the odor similarity of a masked cheese sample to the same sample used as a reference, the odor of the hidden sample was not evaluated as similar to the odor of the reference sample. Moreover, this difference may be explained by the high volatility of extracted compounds, which are very labile.

To conclude, with regard to the sensory profiles of the extracts, the optimal conditions of extraction were suitable for extraction of the volatile compounds of apple even if cooked apple odor was highest for some extracts (Fuji). The intensity marks of extracts are high, whereas the similarity marks are low but acceptable in comparison to the literature.

Conclusion. The dynamic headspace extraction was optimized to extract a maximum number of volatiles and especially volatiles contributing to apple aroma. The optimal extraction

conditions were 40 °C and 70 min of extraction. The addition of saliva increased the extraction of volatiles, but its influence was different for different molecules. New studies will be done to understand better this influence of saliva and its ingredients on the extraction of apple aroma by the dynamic headspace method. We will investigate the influence of salts, α-amylase, and mucins separately.

With regard to the sensory profiles of the extracts, the optimal conditions of extraction were suitable for the extraction of the volatile compounds of apple even if cooked apple odor was highest for some extracts (Fuji). Another study will be done to verify if these observations are also true for Gala apples.

ABBREVIATIONS USED

TQOV, total quantity of odorant volatiles; TQV, total quantity of extracted volatiles; GC-MS, gas chromatography coupled to mass spectroscopy; ANOVA, analysis of variance; LSD, least significant difference; MLR, multiple linear regression.

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