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Flavor-Active Compounds Potentially Implicated in Cooked Cauliflower Acceptance

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The aim of the present study was to determine the flavor-active compounds responsible for the "sulfur" and "bitter" flavors of cooked cauliflower potentially implicated in cauliflower rejection by consumers. Eleven varieties of cauliflower were cooked and assessed by a trained sensory panel for flavor profile determination. Among the 13 attributes, the varieties differed mainly according to their "cauliflower odor note" and their "bitterness". Various glucosinolates were quantified by HPLC and correlated with bitterness intensity. The results showed that neoglucobrassicin and sinigrin were responsible for the bitterness of cooked cauliflower. Application of Dynamic Headspace GC−Olfactometry and DH-GC-MS showed that allyl isothiocyanate (AITC), dimethyl trisulfide (DMTS), dimethyl sulfide (DMS), and methanethiol (MT) were the key odorants of cooked cauliflower "sulfur" odors. Moreover, these volatile compounds corresponded to the main compositional differences observed between varieties. Finally, AITC, DMTS, DMS, MT, sinigrin, and neoglucobrassicin were shown to be potential physicochemical determinants of cooked cauliflower acceptance.

KEYWORDS: Cooked cauliflower; bitterness; sulfur flavor; odor-active compounds; gas chromatography−olfactometry; sensory analysis

INTRODUCTION

Cruciferous vegetables have been identified among other foods as potential contributors of anticarcinogenic compounds to the diet (*1*), focusing considerable interests on their consumption. In France, vegetable producers have noticed a relative decline in cauliflower purchase. A recent survey conducted by the regional fruit and vegetables economic committee (Comité économique régional agricole fruits et légumes, CERAFEL) found evidence that flavor was one of the main reasons some consumers rarely or never purchase cauliflower. Several studies have suggested that certain flavor properties, such as bitterness or the typical skatole aroma, may be considered, at least by some consumers, as undesirable in food and beverage and, then, possibly influencing their consumption habits (*1*, *2*). Bitter taste sensitivity may play, for instance, a key role by reducing intake among individuals who are the most sensitive to the bittering agent of a given food (*3*, *4*). Similarly, it may be speculated that rejection of cooked cauliflower by consumers who eat little or no cauliflower may be partly due to their higher sensitivity to compounds responsible for "undesirable" flavor notes.

Over the past two decades, the fresh market and processing industry have gathered complaints about the bitter taste of some Brussels sprouts cultivars. Because Brussels sprouts are widely consumed in Europe (*5*), most studies searching to identify bittering agents have focused on this vegetable. Two compounds, sinigrin and progoitrin, have been pointed out as the main sources of bitterness. Van Doorn et al. (*5*) have shown that higher concentrations of these two compounds were associated with a sharply smaller number of consumers who report that the product has "good taste". These data strengthen earlier reports that the glucosinolate concentration in raw or cooked *Brassica* foods is the principal barrier against consumer acceptance (*1*, *6*).

Similarly, unpleasant odor arising during cooking as well as during consumption has been considered to be responsible for the low consumption of sauerkraut in the United States. Coleslaw and salad containing cabbage have been reported to present sulfurous aroma and excessive hotness, which impede consumer acceptance (*7*). Among the compounds potentially active in cooked *Brassica* food flavor, certain sulfides such as methanethiol, dimethyl sulfide, and dimethyl trisulfide have often been incriminated in objectionable sulfurous aromas and overcooked off-flavors (*7*−*10*). According to numerous reports, glucosinolate metabolites could also be implicated in hotness and sulfurous aroma. In particular, products of sinigrin hydrolysis such as allyl isothiocyanate and allyl cyanide could play a fundamental role in these perceptions (*11*−*14*), but their exact contribution to flavor has not been clearly elucidated.

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Most studies dedicated to identifying odor-active compounds of cooked *Brassica* vegetables are based on the quantification of volatile compounds recovered in a vegetable extract and their comparison with sensory thresholds compiled in the literature. However, because gas chromatography (GC) detectors are often less sensitive to odorants than the human olfactory system (*15*), there is no guarantee that all of the important flavor compounds will be among the volatiles identified (*16*). GC−olfactometry techniques, when used with an appropriate extraction technique, offer a suitable alternative to overcome such limitations.

The objectives of the present study were, first, to determine the compounds potentially implicated in the flavor of cooked cauliflower with a special interest for bitter and sulfur notes and, second, to identify compounds that may be involved in consumer rejection. We first characterized the flavor properties of 11 selected cultivars of cooked cauliflower. Second, we determined and, when possible, quantified compounds potentially responsible for the main flavor properties of the studied cooked cauliflowers. The potential effect of the odor- and taste-active compounds of the different cultivars on consumer rejection is discussed.

MATERIALS AND METHODS

Materials. Eleven varieties of fresh cauliflower encoded A−K were harvested at the same growth stage from December 1999 until April 2000. They were chosen among the most popular Breton varieties. The 11 varieties were selected among 18 products in order to represent a range of different flavor characteristics according to a preliminary 2 of 5 sensory tests with 13 panelists. After washing and chopping, the florets were blanched in water (3.5 min at 100 °C) and then immediately cooled by immersion in cold water just before freezing. The samples were frozen at −22 °C. After defrosting (12 h, 20 °C), 1 kg of florets of each variety was cooked for 6 min under pressure (65 kPa) in a 10 L pressure cooker (SEB, France) with 0.8 L of mineral water (Volvic, France) salted with 10 g/L NaCl (Merck, Darmstadt, Germany). Two hundred grams of cooked cauliflower was immediately frozen and stored at −80 °C before further GC and high-performance liquid chromagoraphy (HPLC) analysis. The remaining florets were used for sensory analysis.

Sensory Analysis. Just after cooking, 11 varieties of cauliflower were assessed by a trained sensory panel to (i) identify descriptive terms allowing product description, (ii) point out the nature of flavor differences between varieties with a special interest for sulfur and bitter flavor notes, and (iii) select varieties for the further determination of odor- and taste-active compounds. Fifty grams of florets was placed in a 200 mL plastic cup in a polystyrene cup holder. The evaluations were conducted in an air-conditioned room (21 ± 1 °C) under white light. The room was fitted with 10 separated booths in compliance with ISO8589. Thirteen panelists, volunteers from Bretagne Biotechnologie Végétale (BBV; St Pol-de-Léon, France), were selected, first, for their ability to rank bitter or odorant solutions according to their intensity and, second, for their ability to describe and to discriminate different cauliflower varieties. Eighteen 1−1.5 h sessions were dedicated to generating the vocabulary to be used to describe the 11 cauliflower varieties and to train the panelists to use the selected attributes. An initial list of 156 terms describing texture (35), odor (72), aroma (45), and taste (4) was finally reduced to 24 terms by consensus. Odor was defined as the olfactory or trigeminal sensations perceived via the orthonasal way and assessed by smelling the product. Aroma was defined as the olfactory or trigeminal sensations perceived in mouth via the retronasal way. Taste was the gustatory sensation defined by the attributes bitter, salty, sweet, and sour. Attributes chosen for training are listed in **Table 1**. The panelists were trained to quantify the intensity of each descriptor using a 10 cm unstructured linear scale anchored from "no perception" to "strong". The intensity of the reference solution (**Table 1**) was indicated by a mark on the scale. Final sensory profiling was performed by 10 panelists. At each session, 5−6 of the 11 varieties were presented in a monadic way according to a Williams Latin Square design (*20*) to balance report and position effects. Each variety was evaluated twice by the whole panel. During the sessions, the panelists could taste each reference solution. Between samples, they rinsed their mouths with bread and mineral water.

Table 1. Perceived Intensity of the References Used To Define the Terms of the Descriptive Analysis

description	reference	intensity[a]
rubber odor	benzothiazole (IFF, France)	5
mushroom odor	oct-1-en-3-ol (IFF, France)	5
herbaceous odor	coumarine (Aldrich, France)	6.6
green odor	(*Z*)-3-hexenol (IFF, France)	5
potato odor	methional (Aldrich, France)	5
old odor	butyric acid (Aldrich, France)	5
mushroom aroma[b]	paris mushroom macerated in mineral water (Volvic, France)	5
nutty aroma[b]	hazelnut powder (Vahiné, France)	5
potato aroma[b]	mashed potatoes (Mousseline, France)	3.3
hearty aroma[b]	2-methylisoborneol (Aldrich, France)	6.6
green aroma[b]	(*Z*)-3-hexenol (IFF, France)	5
sweet	sucrose	5
salty	sodium chloride	5
bitter	caffeine (Aldrich, France)	5
sour	citric acid (Aldrich, France)	5

[a] Corresponding intensity of the reference (/10). [b] Aroma: retronasal olfactory perception.

Table 2. Gradient Program Used for the RP-HPLC Analysis of Glucosinolates

time (min)	pure water (%)	acetonitrile/water 25:75 v/v (%)	pure acetronitrile (%)
0	96	4	0
1	86.4	13.6	0
10	60	40	0
11	44	56	0
26	4	96	0
31	0	0	100
36	99	0	1
37	96	4	0

HPLC Identification and Quantification of Glucosinolates. For each of the three replicates, freeze-dried florets were crushed in liquid nitrogen to obtain a uniform powder. A quantity of 200 mg was added in a tube and heated for 5 min in a 95 °C water bath to inactivate myrosinase. After the addition of 2 mL of sterile boiled water and 20 μL of internal standard (glucotropaeolin, 20 mM), the tube was heated at 95 °C, for 5 min, cooled in an ice bath, and centrifuged for 10 min at 10000 rpm and 4 °C. Once the supernatant was recovered and placed in an ice bath, 1 mL of boiled water was added to the pellet. The sample was then heated for 5 min at 95 °C and centrifuged to recover a supernatant. A volume of 150 μL of barium acetate (0.5 M) was added to 1 mL of the mixture of the two supernatants. After stirring for 5 s with a vortex, the mixture was deposited on a DEAE-Sephadex A-25 column (Sigma, St Quentin Fallavier, France). A volume of 500 μL of sulfatase at 10 units/mL purified from *Helix pomatia* was added in the column. After a reaction time of 18 h, desulfated glucosinolates were eluted with 2 mL of water. The desulfated glucosinolates were quantified by HPLC on an RP-18 Lichrospher column (LichroCART 250-4, Merck). A volume of 75 μL was injected, and the flow rate was 0.7 mL/min. Separations were carried out using the gradient compositions given in **Table 2**. The glucosinolates were detected at 229 nm with a 996 diode array detector (Waters, Milford, MA). Two injections were performed per replication. Compound identifications were based on (1) comparison with the retention time of pure compounds injected in the same conditions and (2) UV spectrum (*19*). For some compounds, HPLC-mass spectrometry (MS) data were used to confirm the identification.

Dynamic Headspace (DH). After defrosting (12 h, 4 ± 1 °C), ∼25 g of florets was dispersed in distilled water (w/w = 1/4) and

homogenized for 3 min at 19000 rpm with a Polytron PT/MR 2100 (Kinematica, Lucerne, Switzerland). The "cauliflower juice" was stored for at least 2 h at 4 °C before further analysis. The volatile compounds in each cauliflower juice were extracted with a dynamic headspace analyzer (3100 sample concentrator, Tekmar Inc., Cincinnati, OH). After homogenization of the suspension (1 min, 1000 rpm), a 10 mL sample was transferred to an analytical glass tube for immediate analysis. Each sample tube was connected to the apparatus. After 2 min of prepurge, the tube was heated for 8 min at 80 °C and then purged with high-purity helium at a flow rate of 30 mL/min for 10 min. The volatiles were extracted by adsorption to a porous-polymer-adsorbent Tenax trap column (60/80 mesh; 0.25 g; 30 by 0.32 cm; Tekmar Inc.) at 40 °C. This column was heated at 220 °C for 2 min to desorb the volatiles, which were transferred at 150 °C to the head of a capillary column with cryofocusing at −150 °C.

DH-GC-MS. The condensed volatile compounds were analyzed by GC (model 6890; Hewlett-Packard, Avondale, PA) by heating the interface to 180 °C for 1 min and automatic splitless injection onto a nonpolar capillary column (HP-5MS; 30.0 m by 0.25 mm; 0.25 μm film thickness) at a helium velocity of 30 cm·s^{-1}. The oven temperature was held at 5 °C for 5 min and then programmed to rise from 5 to 20 °C at 3 °C·min^{-1} followed by a rate of 5 °C·min^{-1} to 100 °C and then by a gradient of 15 °C·min^{-1} to 150 °C, at which the temperature was maintained for 5 min. The GC column was connected to a mass-sensitive detector (model 6890A quadrupole mass spectrometer; Hewlett-Packard). The GC-MS interface was heated at 280 °C with the actual temperature in the MS source reaching 180 °C. The electron impact energy was set at 70 eV, and data were collected in the range of 29−300 atomic mass units at a scan rate of 1.68 scans·s^{-1}. The same analytical procedure was performed in triplicate for each of the 11 cooked cauliflower varieties. A 10 mL blank of pure water was analyzed according to the same procedure before and after each variety. Compound identification was based on (1) comparison of retention indices (RI) (*17*) with RI published or obtained for pure compounds in our laboratory, (2) mass spectra (MS spectra databases NBS75K and Wiley 275L), and (3) odor properties. In some cases, the detection of a compound (similar RI values) in all of the varieties allowed us to confirm its identification in the sample when its quantity was too low.

GC-MS of a Dichloromethane Extract. When RI and odor perception suggested the presence of a compound not properly detected in the DH extract of any variety, a concentrated dichloromethane extract of the cauliflower juice was analyzed. One hundred milliliters of cauliflower juice of variety J was prepared according to the method described for DH samples. After centrifugation of 100 mL of the juice (14000 rpm, 45 min, 4 °C), 84 g of supernatant was recovered. Salt (8.4 g of NaCl) was added to the supernatant, and the blend was stirred for 15 min. The solution was extracted three times with dichloromethane (successively 10, 5, and 5 mL). The organic layers were pooled, dried over anhydrous sodium sulfate, and concentrated to 0.5 mL under a light stream of pure nitrogen. The extract was stored at −80 °C until further GC-MS analysis. The GC-MS system was the same as previously described for DH-GC-MS except for the injector and oven program: 1 μL splitless injection (30 s valve delay); injector temperature, 250 °C; oven programmed from 40 to 250 °C at a rate of 3 °C·min^{-1} with initial and final hold times of 5 and 10 min; helium velocity, 30 cm·s^{-1}. Standard MS spectra databases (NBS75K, Wiley 275L) were used for compound identification.

DH-GC—Flame Ionization Detection—Olfactometry (DH-GC-FID-O). The GC-FID-O system consisted of the same GC model as for DH-GC-MS. The conditions of injection and separation of the volatile compounds were also exactly the same. At the end of the column, the effluent was split 1:1 between an FID maintained at 250 °C and a Sniffer 9000 sniffing device equipped with a humidified air makeup (heated transfer line temperature = 150 °C, humid air = 80 kPa, Brechbühler SA, Switzerland). To determine potentially odor-active compounds of the cooked cauliflower, the nasal impact frequency (NIF) method (*15*) was performed. According to Pollien et al. (*15*), who recommended 8−10 subjects, 12 panelists were selected among volunteers in the laboratory. The sniffing sessions were conducted in an air-conditioned room (20 ± 1 °C). Sniffing was divided into two parts to avoid lassitude (*15*). The sessions were planned in such a way that each subject participated in the sniffing of both parts, 1 h apart. Panelists recorded the perception of an odor by pressing a button as long as the compound could be smelled. Moreover, each panelist was encouraged to describe the odor of each compound detected. The square signal was registered by an HP Chemstation (Hewlett-Packard). GC-O analysis was performed with two cauliflower varieties (E and J). The 12 aromagrams of a given sample were summed, yielding a total aromagram. Detection frequency of <33% (<5/12 subjects) was considered to be noise (*18*). Odor zones were determined as the maximal width of each peak. For each odor zone, a detection frequency was calculated as the percentage of panelists who detected an odor. Similarly, a quotation frequency was calculated for each odor zone as the percentage of panelists who used similar terms to describe an odor. To point out the importance of a given note for a subject able to detect it at the sniffing port, an index named " relative quotation frequency" equal to the ratio between quotation frequency and detection frequency was calculated for each odor-active compound. The molecule assumed to be responsible for an odor was chosen among the compounds identified at DH-GC-MS according to the following criteria: (1) RI was close to the detection zone of the panelists and (2) the terms used to describe the molecule were close to those reported by the literature. In some cases, injection of pure compounds allowed us to strengthen the identity of a potential odor-active compound.

Data Analysis. Sensory analysis data were recovered and processed with the Tastel software version 2000 (ABT informatique, Rouvoy sur Marne, France). Two-way analysis of variance (product, subject) was performed on the sensory data set according to the model attribute = product + subject + product × subject, with subject treated as a random effect. When significant differences were evidenced ($P < 0.05$), sample mean intensities were compared using the least significant difference multiple comparison test (LSD). Principal component analysis was performed with Statbox version 2.5 (Grimmer Logiciels, Paris, France) on the analytical data to demonstrate composition differences among varieties. Stepwise multiple linear regression was performed with the SAS system, release 6.12 (SAS Institute Inc., Cary, NC), using proc REG with the stepwise option.

RESULTS AND DISCUSSION

Descriptive Analysis. The first objective of the descriptive analysis was to identify terms enabling product description. The gustatory dimensions used to characterize the products were bitter, salty, sour, and sweet. Aroma and odor were described by several common terms such as mushroom, green, potatoes, pungent, and a typical "cauliflower" note. However, some attributes such as rubber, herbaceous, and old were quoted exclusively for odor, whereas other attributes were used to describe only aroma (nutty, earthy). These qualitative and quantitative differences observed between aroma and odor perceptions have already been reported and may be due to differences in odorant release conditions associated with each of the two olfactory perceptions (*21*) and interaction between taste and retronasal olfaction (*22*). Finally, bitter taste (**Table 3**) and cauliflower aroma (data not shown) presented the highest average intensities (3.9 and 3.7, respectively), demonstrating that these two characteristics were key flavor attributes of cooked cauliflower.

The output of two-factor ANOVA (product, subject) showed a significant product effect for seven attributes (**Table 3**): cauliflower and pungent odor, nutty and pungent aroma, and sweet, bitter, and sour tastes. These attributes reflected sensory differences between varieties that may determine consumer choice. Considering the gustatory attributes, it was noteworthy that the varieties F, G, and H were bitterer than A, B, and D but less sweet. There was a strong negative correlation ($r = -0.92$) between bitterness and sweetness. This reciprocal suppression phenomenon has already been demonstrated in psychophysical studies (*23*). Moreover, Van Doorn et al. (*5*)

Table 3. Odor, Aroma, and Taste Differences among 11 Cooked Cauliflower Varieties[a]

	odor		aroma		taste		
variety	cauliflower	pungent	nutty	pungent	sweet	bitter	sour
A	2.8ab	3.2b	2.0a	1.8a	3.9c	2.8a	1.6a
B	3.2abc	2.2a	1.9a	1.5a	3.7c	3.0ab	1.6a
C	2.5a	3.2b	2.6a	3.1bc	3.0bc	4.1cde	1.9ab
D	3.0abc	3.5b	2.1a	2.2ab	3.6c	3.2abc	1.7a
E	2.7ab	4.0b	4.3a	2.2ab	3.0bc	4.3de	1.9abc
F	3.2bc	3.8b	2.3a	4.3d	1.6a	5.6f	2.8cd
G	3.1abc	3.7b	2.0a	4.0cd	2.1ab	4.2de	3.1d
H	3.5cd	3.9b	2.5a	3.7cd	2.1ab	4.8ef	2.4abcd
I	3.9d	3.5b	2.4a	2.1ab	3.1bc	3.4abcd	1.8a
J	4.1d	3.7b	2.2a	2.4ab	3.0bc	3.4abcd	1.9ab
K	3.2abc	3.4b	2.4a	3.0bc	3.3c	4.0bcde	2.7bcd
mean[b]	3.2	3.5	2.4	2.8	2.9	3.9	2.1

[a] For each variety (A–K) and each attribute, two replicates of 10 panelists' evaluation are averaged. For each attribute, means with the same letters do not differ significantly (5%) according to the LSD test. Odor is the olfactory or trigeminal sensation perceived via the orthonasal way and assessed by smelling the product. Aroma is the olfactory or trigeminal sensation perceived in the mouth via the retronasal way. Taste is the gustatory sensation defined by the attributes bitter, salty, sweet, and sour. [b] Mean intensity value for the 11 varieties.

mentioned several annual reports from various U.K. institutions where bitterness and sweetness owning to different cultivars of Brussels sprouts were always significantly negatively correlated ($r^2 = 0.51-0.88$, $P < 0.05$). Thus, the gustatory potential of bitter stimuli might be modulated by compounds responsible for sweet taste.

Varieties F, G, and H were distinguished from most varieties (A, B, D, E, I, and J) for their pronounced pungent aroma, but pungent aroma was poorly correlated with pungent odor. Moreover, the fact that pungent "aroma" was strongly correlated with sweetness, bitterness, and sourness ($r = -0.92$, 0.88, and 0.89, respectively) may reflect the difficulty for the panelists to distinguish pungency, bitterness, and sourness sensations. Consequently, pungency differences have to be interpreted with caution. Whereas varieties I and J were characterized by a more intense cauliflower odor than eight of the nine other varieties (3.9 and 4.1, respectively), variety E was differentiated for its intense nutty note (4.3).

Two varieties (J and E) were chosen for further GC-O experiments because of differences in their olfactory attributes and especially their cauliflower odor. This attribute can be assimilated with the sulfur note frequently described, with bitterness, as objectionable for cruciferous vegetables when it is too pronounced (*1*, *7*). It is a possible determinant of consumer choice between varieties and perhaps also of the rejection behavior of nonconsumers. Variety J was selected because it exhibited the most intense cauliflower odor. Variety E was retained for its weak "cauliflower" odor associated with a particularly strong nutty aroma.

Determination of Compounds Implicated in Cauliflower Bitterness. By analogy with the results of studies performed on other cruciferous vegetables (*1*, *5*, *24*), it was hypothesized that the bitterness of cooked cauliflower could be due to its content in bitter glucosinolates. Levels of glucosinolates for the 11 varieties (**Table 4**) were far greater than the values reported by Sones et al. (*26*), probably because these authors used a GC technique that underestimated the glucosinolate contents. In contrast, our data were in accordance with the results obtained by Khushad et al. (*26*) using HPLC techniques. The total content in glucosinolates differentiated cauliflower varieties. Total glucosinolate contents of varieties F, G, and J were >3 times that of variety A. These differences were mainly due to variations in aliphatic glucosinolate content (glucoiberin, progoitrin, sinigrin, and glucoiberverin) and especially sinigrin, the concentration of which varied from 1.69 (A) to 7.99 g/kg of dry matter (F).

The highest correlation coefficients between glucosinolate content and bitterness intensity was observed for neoglucobrassicin ($r = 0.70$) and sinigrin ($r = 0.56$). The model obtained with stepwise multiple linear regression confirmed a significant contribution only for neoglucobrassicin (partial $R^2 = 0.49$, P

Table 4. Glucosinolate Contents of 11 Varieties of Cooked Cauliflower[a]

variety	gluco-iberin	progoitrin	sinigrin	gluco-iberverin	gluco-brassicin	4-methoxy-glucobrassicin	neogluco-brassicin	aliphatic glucosinolates	indolic glucosinolates	total glucosinolates
A	1.22	0.22	1.69	0.66	1.63	0.04	0.20	3.79	1.87	5.66
	0.32	*0.08*	*0.19*	*0.63*	*0.38*	*0.00*	*0.16*	*0.45*	*0.52*	*0.55*
C	1.20	0.84	5.88	2.85	2.19	0.15	0.54	10.77	2.90	13.68
	0.22	*0.06*	*0.35*	*1.14*	*0.23*	*0.05*	*0.25*	*1.42*	*0.48*	*1.16*
E	1.81	0.97	5.10	2.36	3.04	0.17	0.40	10.24	3.63	13.86
	0.79	*0.20*	*0.59*	*0.81*	*1.05*	*0.07*	*0.24*	*1.47*	*1.33*	*2.32*
D	2.41	0.78	4.70	3.93	2.36	0.08	0.37	11.82	2.81	14.63
	0.50	*0.30*	*1.44*	*2.73*	*0.83*	*0.02*	*0.23*	*4.02*	*0.63*	*4.65*
H	2.40	1.45	4.38	2.14	3.62	0.17	0.53	10.37	4.32	14.69
	0.82	*0.29*	*0.15*	*1.14*	*0.81*	*0.15*	*0.18*	*0.79*	*0.78*	*1.52*
B	2.84	0.74	5.29	2.02	3.57	0.19	0.42	10.88	4.18	15.07
	0.82	*0.22*	*0.31*	*1.22*	*0.74*	*0.02*	*0.12*	*0.93*	*0.85*	*1.32*
I	2.75	1.97	6.28	1.13	2.66	0.16	0.42	12.14	3.24	15.38
	0.40	*0.48*	*0.46*	*0.28*	*0.51*	*0.04*	*0.28*	*0.34*	*0.79*	*1.12*
K	3.79	1.85	6.24	0.79	1.79	0.04	0.10	12.66	1.98	14.64
	0.82	*0.25*	*0.93*	*0.47*	*0.41*	*0.01*	*0.05*	*1.44*	*0.49*	*1.89*
J	4.77	2.51	6.25	1.54	2.26	0.02	0.10	15.07	2.40	17.47
	1.90	*0.43*	*1.36*	*0.82*	*0.09*	*0.01*	*0.15*	*2.88*	*0.26*	*3.13*
G	2.91	1.76	6.47	2.93	2.78	0.04	0.74	14.07	3.55	17.63
	0.52	*0.18*	*0.80*	*0.56*	*0.29*	*0.02*	*0.10*	*1.06*	*0.24*	*0.88*
F	2.57	1.73	7.99	2.51	2.66	0.18	0.94	14.80	3.78	18.58
	0.28	*0.61*	*1.04*	*0.73*	*0.06*	*0.03*	*0.21*	*1.40*	*0.15*	*1.55*
r bitter[b]	–0.04	0.34	0.56	0.25	0.30	0.39	0.70	0.44	0.47	0.51

[a] Varieties are listed by increasing content of total glucosinolates. For each variety (A–K) and each glucosinolate quantified, three replicates are averaged. Data are expressed in $g \cdot kg^{-1}$ of dry matter of cooked product. Confidence interval (%) is reported in italic type ($P < 0.05$). [b] Correlation coefficient between content in glucosinolates and bitterness of the varieties.

= 0.016). These results indicated that the concentration of these particular compounds was not sufficient to fully explain bitterness. Other parameters such as mixture effect involving sweet stimuli may also have contributed to the bitter taste. To our knowledge, no published data have demonstrated the bitter taste of neoglucobrassicin. It would be helpful to investigate this point to determine its potential contribution to the bitterness of cooked cauliflower. In contrast, the bitterness of sinigrin and its role in the taste of cruciferous vegetable were in accordance with previous studies performed on Brussels sprouts and cabbage (*5*, *24*). When expressed in liters of cooked cauliflower water content, the concentration of sinigrin varied from 0.12 to 0.56 g/L. In all cases, this value was greater than the sinigrin threshold of 0.106 g/L of pure water given by Drewnovski and Gomez-Carneros (*1*). By analogy with Brussels sprouts, it seemed to be realistic that sinigrin would be responsible for the bitter taste of cooked cauliflower.

Relationship between DH-GC-O Olfactory Notes and Descriptive Analysis. The results of GC-O analysis are summarized in **Table 5**. Fifty-eight compounds were tentatively identified with GC-MS, and the identities of 21 of them were confirmed by injection of pure standards. Thirty-three odor compounds were perceived by at least 5 of the 12 panelists, and 28 of them were identified using GC-MS, RI, olfactory notes, and standards. Due to their concomitant detection and their sensory proximity, terms quoted by the sniffers were gathered together in the following odor families: "sulfur" (cabbage, cauliflower, garlic, onion, sulfur, ripened cheese), "green" (cut grass, grass, green), "citrus-fruit" (citrus fruit, orange, orange peel), "mushroom" (moldy, mushroom), "floral" (daisy, floral), "butter" (butter, caramel), "earthy" (earthy, undergrowth), "toasted" (burnt, toasted, roasted), "cooked potatoes", "pungent", "fatty" (fatty, floor cloth, rancid), and "crushed bug." Most of these notes corresponded to the main descriptors chosen by the trained panel to describe aroma and odors of the cooked product. Nevertheless, some odors such as "butter", "fatty", "citrus-fruit", "toasted", and "crushed bug" were perceived only in GC-O and were not chosen as descriptive terms for the sensory descriptive analysis of cooked cauliflowers. This could be explained by a higher concentration of the corresponding compounds in the chromatographic eluate compared with that occurring in the product. The DH method chosen to recover the volatile may have overconcentrated some compounds. Moreover, masking effects between compounds in mixture could have occurred in sensory assessment of the entire product. Finally, psychophysical studies have shown that the human olfactory system was unable to discriminate more than four or five olfactory notes in mixture (*27*). This observation implies that some of the single odors perceived in GC-O for separated compounds could have been obscured in descriptive analysis of the whole product because they were less distinguishable than the four or five dominant flavor notes. However, despite their apparent minor contribution, it is probably necessary to take into account the participation of these compounds in the olfactory background in order to fully explain odor or aroma.

Most of the odor compounds were detected in varieties E and J with a high level of detection. However, for some compounds such as methyl ethyl sulfide, the quotation frequency differed between the two varieties, and for others, the differences were quoted by at most three panelists. Taking into account the lack of consensus among panelists, it was difficult to conclude which compounds were responsible for the odor/aroma differences pointed out by the descriptive analysis. Consequently, although the NIF technique did not directly point out the origin of the olfactory differences identified by descriptive analysis, identification of compounds responsible for the olfactory notes detected and quoted with the NIF method did provide an explanation of the main "cooked cauliflower" descriptors, common to the two varieties, analyzed with GC-O.

Compounds Potentially Responsible for the Main "Sulfur" Odor Notes. DH-GC-O detected 11 "sulfur" zones for varieties E and J. Detection frequencies indicated that 9 of them were perceived by 58% of the sniffers in one of the two varieties. The relative quotation frequency indicates the proportion of the sniffers detecting an odor who properly quoted the main attributes. With these two indices, five main sulfur zones could be distinguished among the nine detected by the sniffers and four of them were associated with identified compounds.

Methanethiol (MT). Identified at an RI of 464, this compound described as "putrid, fecal-like aroma" (*28*) or as "cooked cabbage" (*29*) was responsible for the "sulfur, cooked cabbage" note perceived by the sniffers. In cruciferous vegetables, it is derived from the breackdown of *S*-methyl-L-cysteine sulfoxide (SMCSO). It is considered to be one of the major sulfur odorants in numerous food products because of its low flavor threshold, 0.02 ppb in water according to Lindsay and Ripe (*28*), and also because it is a precursor of numerous sulfur compounds (*30*, *31*). This compound, present in various cruciferous vegetables, has been reported as responsible for the off-flavor of fresh products stored under anaerobic atmospheres (*9*, *10*). The relative quotation frequency value reached approximately 49% for variety E and 40% for variety J. Thus, although the contribution of MT to the odor of cooked *Brassica* has often been considered to be questionable because of its high volatility [boiling point of 6 °C according to Forney et al. (*9*)], our data suggest that it may contribute significantly to the sulfur note of the two varieties.

Dimethyl Sulfide (DMS). Described as "cauliflower" (*29*), its presence at an RI of 515 explained the sulfur note perceived in variety E, where its relative quotation frequency reached 58%. Often reported to account for a very high proportion (~34%) of total volatiles generated in cooked *Brassica* vegetables (*13*), where it is formed from *S*-methylmethionine (*12*), its relatively weak detection threshold value [0.3 ppb in water according to Shankaranarayana et al. (*30*)] tends to confirm its potential contribution to the cauliflower odor of the two varieties.

Allyl Isothiocyanate (AITC). Identified at an RI of 887, its "black mustard-like and pungent" note was responsible for the odor detected by 67% of the sniffers in variety E and 58% in variety J. The absence of consensus for its detection may come from known interindividual differences in sensitivity for this compound (*32*). Moreover, its high relative quotation frequency indicated that most sniffers detecting the compound were able to characterize it. Despite its quite high detection threshold value [375 ppb in water according to Buttery et al. (*3*)], the present study suggested that this compound, formed from sinigrin (*14*), is a potential odor-active compound of cooked cauliflower. This observation agreed with results obtained on other cooked *Brassica* vegetables (*13*, *30*).

Dimethyl Trisulfide (DMTS). Identified at RI = 954, this product of SMCSO breakdown (*31*) was responsible for the odor perceived by 92% of the sniffers and described as "sulfur, cauliflower, cabbage" with a relative quotation frequency reaching 46% in variety E and 63% in variety J. This compound was present in most varieties at a quantity just detectable with our DH-GC-MS device. Nevertheless, its high relative quotation frequency may be easily explained by its very low odor

Table 5. Odor Activity of Volatile Compounds Identified in Cooked Cauliflower

RI[a]	compound	method of identification[b]	odor quoted by the sniffers	dominant odor[c]	detection frequency		relative quotation frequency[d]	
					var E	var J	var E	var J
464	methanethiol (MT)	1–4	sulfur, cooked cabbage	sulfur	67	83	49	40
506	propanal	1	solvent		100	92	25	27
510	2-propanol	1						
515	dimethyl sulfide (DMS)	1–4	cabbage, cooked cauliflower	sulfur	100	83	58	10
567	2-methyl-2-propenal	1						
595	butanal	1, 2		pungent				
595	2,3-butanedione	1–4	buttery, caramel	butter	100	100	58	58
601	2-butanone	1, 2						
603	2-methylfuran	1, 2						
606	3-penten-2-one	1						
609	methylethyl sulfide (MES)	1–4	sulfur, garlic	sulfur	100	100	42	0
623	tetrahydrofuran	1, 2						
623	2-butenal	1						
658	allyl cyanide (AC)	1–4		sulfur				
670–680	unknown	3	sulfur, garlic, onion	sulfur	100	100	42	75
683	2-methyl-2-propenenitrile (2-M-2-P)	1		sulfur				
684	1-penten-3-one	1		pungent				
686	1-penten-3-ol	1	butter	butter				
696	pentanal	1, 2		pungent				
700	2-ethyl furan	1, 2						
701	2,3-pentanedione	1–4	buttery	butter	83	100	70	33
711	methyl thiocyanate (MTC)	1, 2	sulfur	sulfur				
738	dimethyl disulfide (DMDS)	1–4	cabbage, sulfur, ripened cheese	sulfur	50	75	8	0
758	3-methyl-2-butenal	1						
762	methylbenzene	1						
800	octane	1						
805	hexanal	1–4	cut grass	green	100	100	67	67
812–829	unknown	3	burnt, toasted, roasted	toasted	100	100	25	50
856	2-hexenal	1	rancid, fatty	rancid, fatty				
858	ethylbenzene	1, 2						
866	1,3-dimethylbenzene	1						
874	1-cyano-2,3-epithiopropane (1C2,3ETP)	1		sulfur				
887	allyl isothiocyanate (AITC)	1–4	sulfur, garlic, pungent	sulfur	67	58	93	57
888	1,2/1,4-dimethylbenzene	1						
900	nonane	1						
902	heptanal	1–4	citrus fruit, fatty, rancid	rancid, fatty	100	92	25	19
909	methional	2–5	cooked potatoes	potatoes	100	100	50	33
951	2-heptenal	1	green	green				
946	butyl isothiocyanate (BITC)	1	sulfur, green, pungent	sulfur	42	67	41	25
954	dimethyl trisulfide (DMTS)	1–4	sulfur, cauliflower, cabbage	sulfur	92	92	46	63
958	1-ethyl-3-methylbenzene	1						
964	β-pinene	1						
975	1-octen-3-ol	1–4	mushroom	mushroom	92	92	73	54
978	1-butene 4-isothiocyanate	1		sulfur				
980	1-octen-3-one	1–4	mushroom	mushroom	92	92	73	54
985	1,2,4-trimethylbenzene	1						
988	1,5-octadien-3-ol	1	undergrowth, earthy	earthy	92	92	19	9
1001	ethyl hexanoate	1						
1004	3-carene	1–4	citrus fruit, orange peel	citrus fruit	83	92	60	46
1004–1008	unknown	3	floral	floral	100	100	17	25
1009	2,4-heptadienal	1–4	crushed bug, nutty					
1015–1020	unknown	3	floral, daisy	floral				
1022	1-ethyl-2,4-dimethylbenzene	1						
1025	D-limonene	1–4	citrus fruit	citrus fruit	92	75	9	23
1040	3-octen-2-one	1	crushed bug, nutty					
1060	2-octenal	1–4	green	green	92	92	27	0
1076	3,5-octadien-2-one	1	mushroom	mushroom	83	83	30	10
1085	4-ethyl-1,2-dimethylbenzene	1						
1091	4-(methylthio)butenenitrile	2–5		sulfur				
1108	nonanal	1–4	green, citrus fruit	green	92	92	19	19
1110–1140	unknown	3	sulfur, onion, ripened cheese	sulfur	75	75	44	33
1204	decanal	1–4	green, citrus fruit	green	83	92	30	27

[a] Retention index on HP-5MS column. [b] 1, tentative identification using mass spectrometry and MS databases; 2, coincidence between the calculated retention index and known retention index of pure compound; 3, coincidence between odor smelled by the sniffers and known odor of the pure compound; 4, injection of pure compound; 5, compound identified in a dichloromethane extract of cooked cauliflower. [c] Most frequently quoted odor family. [d] Ratio between quotation frequency and detection frequency (%).

threshold [0.01 ppb in water according to Hansen et al. (*10*)]. In contrast with the other sulfur compounds previously mentioned, the higher relative quotation frequency reached in variety J for DMTS gives a possible explanation of the higher cauliflower odor of this variety (**Table 3**). This result confirms the conclusions of previous studies presenting DMTS as a key

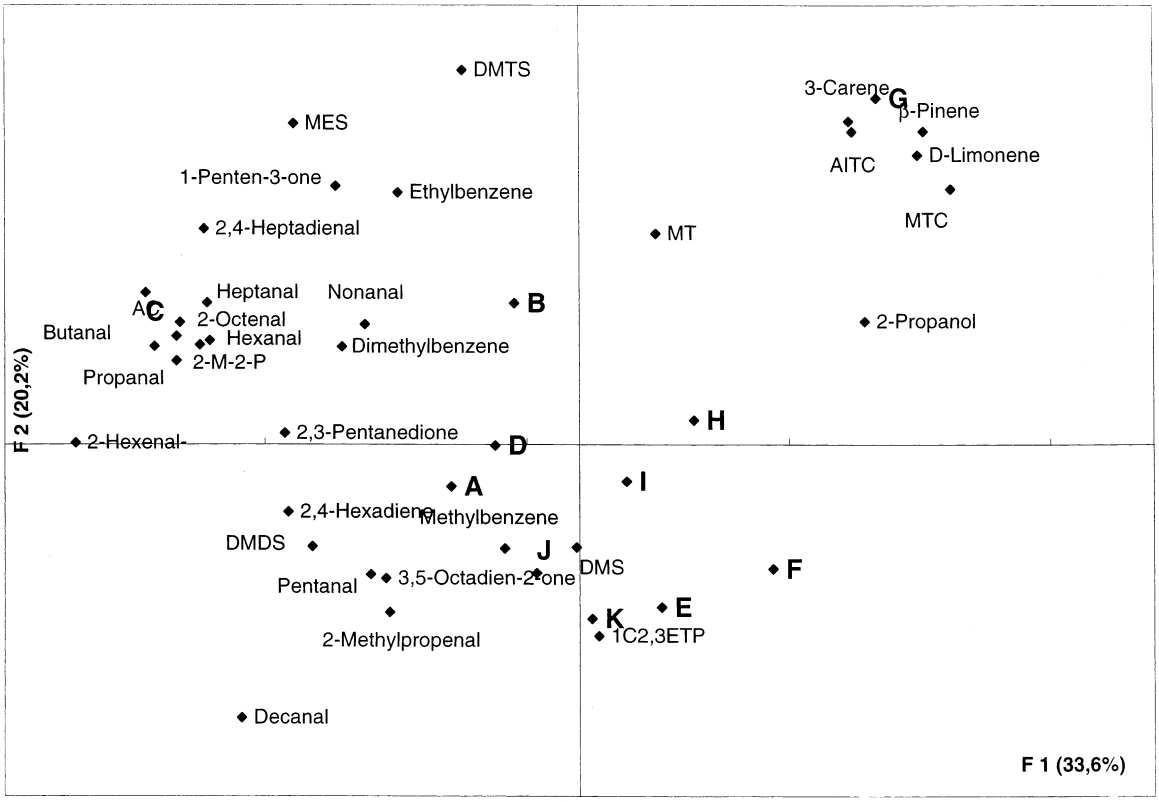

Figure 1. First factorial map of PCA (11 cooked cauliflower varieties, 35 quantified volatile compounds). Names of the compounds corresponding to the abbreviations are given in **Table 5**. Cauliflower varieties are coded with letters (A–K).

compound of the flavor of cooked *Brassica* vegetables (*8*, *11*). Moreover, Chin and Lindsay (*31*) considered this compound to be responsible for the sulfurous off-flavor in cruciferous vegetable foods and, especially, one of the main determinants of consumer preference for sauerkraut.

Other "sulfur" notes were detected by, at least, some of the panelists. Methyl ethyl sulfide, dimethyl disulfide, and butyl isothiocyanate could be responsible for the odor perceived at coincident retention indices. These compounds have already been identified by Buttery et al. (*33*) in cooked *Brassica*. Nevertheless, their weak relative quotation frequency suggests that their contribution to the sulfur note of the whole cooked product was probably weak. Moreover, methyl methanethiosulfinate was not identified in the studied varieties. This compound, which was already identified in sauerkraut and in macerated Brussels sprouts, could participate in their typical sulfur odor due to its sauerkraut sulfur note (*31*). Because thiosulfinates are known to decompose on attempted GC analysis (*34*), it is probable that our analytical device did not reveal their presence.

Compounds Responsible for Other Odor Notes. *"Green" Odor.* The large peak of hexanal at RI = 805 gave a valuable explanation to the odor note perceived simultaneously by 100% of the sniffers and described as "cut grass" by 67% of them in the two varieties. This high quotation frequency suggested a high odor-activity value of hexanal (*35*), which is consistent with its relatively low threshold value [4.5 ppb in water according to Hansen et al. (*10*)]. This observation is in agreement with previous studies showing that hexanal, characterized by a "green, grassy" odor note, is one of the key odor compounds of fresh broccoli florets (*10*, *36*). Even though 2-octenal, nonanal, and decanal may have also contributed, hexanal was probably the main compound responsible for the green note of the cooked product.

"Butter" Odor. A strong odor, detected by 100% of the sniffers and described as "butter, caramel" by 58% of them, appeared at around RI = 595. The presence of ion 86 associated with an overly abundant ion 43 in the spectrum of butanal indicated its coelution with 2,3-butanedione, well-known for its butter, caramel odor note (*37*) and with the same retention index on DB-5 GC capillary columns (*38*). Injection of pure 2,3-butanedione confirmed its participation in the butter odor note occurring simultaneously. Additionally, an intense butter note was also perceived around RI = 700. Identified in the same odor zone (RI = 701) and known for its odor of butter (*29*), 2,3-pentanedione may then contribute together with 2,3-butanedione to the background flavor of the cooked cauliflower.

"Citrus-fruit" Odor. An intense note was detected by at least 83% of the sniffers at RI = 1004 corresponding to the elution of 3-carene. Described as "citrus fruit, orange peel" the relative quotation frequency of this odor reached 60% in variety E. At RI = 1025, another citrus-like note was explained by the elution of D-limonene. Buttery (*33*) mentioned its presence in fresh broccoli, cabbage, and cauliflower, but its actual contribution to the flavor of these products has, to our knowledge, never been proposed. Additionally, heptanal, nonanal, and decanal could also contribute to this note and, then, to the background flavor of cooked cauliflower.

"Mushroom" Odor. A mushroom odor was perceived by 92% of the sniffers in the two varieties. Its relative quotation frequency reached 73% for variety E. Identified at a corresponding RI, 1-octen-3-ol (RI = 975) and 1-octen-3-one (RI = 980) are known for their characteristic mushroom note and for their contribution to this flavor attribute in mold-ripened cheese such as Camembert (*29*). Their occurrence probably mainly explained the mushroom descriptor chosen by the trained panelists to describe cooked cauliflower. Otherwise, GC-O results suggest that 3,5-octadien-2-one, a compound already

identified in the broccoli (*10*), could be responsible for the mushroom note perceived by the sniffers at RI = 1076. Even though its contribution was limited compared to 1-octen-3-ol and 1-octen-3-one, this ketone could also contribute to the global mushroom note of cooked cauliflower.

"Potato" Odor. At an RI of 909, 100% of the sniffers detected an odor described as "cooked potatoes" by 50% of them in variety E. Whatever the variety investigated, it was impossible to identify the compound potentially responsible for this odor, probably because its concentration was too low in the flavor extract obtained by dynamic headspace analysis. By concentrating a dichloromethane extract of variety E under a slight nitrogen flow, it was possible to identify methional, known for its "potato" odor and for its low detection threshold [0.2 ppb in water according to Guadagni et al. (*39*)]. According to Chan and Reneccius (*40*), its formation via a Strecker reaction could explain its frequent occurrence in cooked food. Moreover, its retention index of 911 on a DB-5 column (*38*) confirmed the probable contribution of methional in the potato note of the cooked cauliflower flavor.

"Earthy" Odor. An "undergrowth, earthy" flavor note was described by some sniffers at RI = 988, just after the mushroom note associated with the elution of 1-octen-3-ol and 1-octen-3-one. Whatever the variety, the relative quotation frequency was <20%. However, the tentative identification at a corresponding retention index of 1,5-octadien-3-ol, described by Molimard et al. (*29*) as "earthy, geranium", may give sense to these descriptions. Additionally, its relatively low quotation frequency could be partly explained by its quasi-coelution with a persistent and perhaps dominant mushroom note. Finally, this compound could be responsible for the earthy attribute chosen by the trained panelists to describe the flavor of cooked cauliflower.

"Toasted" Odor. One hundred percent of the sniffers perceived an odor described as "burnt, toasted bread and roasted coffee" by 50% of them in variety J at RI = 812. However, the compound responsible for this note was not identified. Because this attribute was not selected by the panelists to describe the product, it may be hypothesized that the "toasted" odor-active compound plays a secondary role in the flavor of cooked cauliflower.

Discriminative Compounds. The use of GC-O allowed the identification of the odor-active compounds potentially responsible for the main flavor attributes of cooked cauliflower. The high sensitivity of some individuals for some of these volatile components may determine their rejection of the corresponding food. Moreover, differences of concentration between products could explain differences of consumer preference. To identify the molecules potentially responsible for this hedonic discrimination, PCA was performed to distinguish the 11 varieties according to their content in quantifiable volatile compounds. The first factorial map, which represents 53% of the data variance, enabled differentiation between varieties G and C (**Figure 1**). Considering more exclusively the odor-active compounds, variety G was characterized by a higher content in AITC, DMTS, and terpenes such as D-limonene and 3-carene. It is noteworthy that variety G was also distinguished by a higher concentration in glucosinolates, especially sinigrin (**Table 4**). Variety C was characterized by a higher content in allyl cyanide (AC) and aldehydes such as hexanal and 2-octenal. Although the green note did not enable cauliflower discrimination, its higher perceived intensity in variety C could be related to its relatively high hexanal and 2-octenal contents. The third factorial axis (14.7% of variance) allowed variety K to be discriminated for its DMS and, to a lesser extent, its MT contents. This quantitative comparison between varieties suggests that the most potent odor-active compounds according to GC-O were also part of the main discriminative components of cooked cauliflower varieties.

Thus, AITC, DMTS, and, to a lesser extent, DMS and MT can be considered together with sinigrin and neoglucobrassicin as potentially physicochemical determinants of consumer rejection for cooked cauliflower. The next step of our research will now consist in conducting a consumer study to validate the existence of a relationship between consumer sensitivity to the latter compounds and consumer habits concerning cooked cauliflower.

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