A SENSORY, CHEMICAL AND CONSUMER STUDY OF THE PEPPERY TYPICALITY OF FRENCH GAMAY WINES FROM COOL-CLIMATE VINEYARDS

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Abstract

Aim: Within the protected designation of origin (PDO) Côtes d'Auvergne, Gamay N wines express unique peppery notes that may reflect high levels of rotundone. We investigated the typicality of these wines by determining their sensory, chemical and consumer profiles.

Methods and results: Twenty-one Gamay N wines from the 2013 vintage from four French wine-growing areas were assessed by a trained sensory panel (n = 8). Principal component analysis and hierarchical clustering of olfactory data were used to describe differences among regions and to select four wines for a consumer study (n = 87). Gamay N wines from Auvergne had more intense peppery notes and higher rotundone concentrations, two characteristics that showed a significant positive correlation. The large variability in rotundone among the 12 wines from Auvergne was attributed to ethanol content, which was correlated to the rotundone levels in the wines. Those who appreciate wines with a peppery sensory profile were generally managers and professionals who are willing to pay more for a bottle of wine.

Conclusion: There were differences in sensory profile and rotundone concentrations in Gamay N wines from cool-climate vineyards. We also identified the consumption profile of those who appreciate peppery wines.

Significance and impact of the study: Our results provide a scientific foundation for Auvergne grape growers to promote the typicality of their wines. This research also identifies the key elements for developing the Côtes d'Auvergne wine range and adapting products to consumer profiles.

Key words: typicality, Gamay N, rotundone, peppery wine, cool climate

Résumé

Objectif: Au sein de l'Appellation d'Origine Protégée (AOP) Côtes d'Auvergne, les vins de Gamay N expriment des caractéristiques poivrées uniques qui pourraient être imputables à la présence de hauts niveaux de rotundone. Afin d'étudier cette typicité, un travail de recherche incluant une étude sensorielle, analytique et consommateur a été mené.

Méthodes et résultats: Vingt-et-un vins de Gamay N du millésime 2013 en provenance de quatre régions viticoles françaises ont été évalués par un jury expert (n = 8). L'analyse en composantes principales et la classification hiérarchique ascendante des données de l'examen olfactif ont été utilisées afin de décrire les différences entre les régions et de sélectionner quatre vins pour participer à deux études consommateur (n = 87). Les vins de Gamay N d'Auvergne présentent des notes poivrées et des concentrations en rotundone supérieures, et une corrélation significative positive a pu être établie entre ces deux paramètres. Afin d'expliquer l'importante variabilité de concentration en rotundone au sein des 12 échantillons auvergnats, la teneur en éthanol des vins a été identifiée comme une variable clé, significativement corrélée au niveau de rotundone dans les vins. Les consommateurs appréciant les vins poivrés s'apparentent à des cadres et possèdent un budget élevé.

Conclusion: Nos résultats ont permis de mettre en évidence des différences de profil sensoriel et de concentrations en rotundone dans les vins de Gamay N en provenance de vignobles au climat frais. Les résultats acquis ont également permis d'identifier le profil de l'amateur de vins poivrés.

Signification et impact de l'étude: Nos résultats sont susceptibles d'aider les producteurs auvergnats à promouvoir la typicité de leurs vins. Ce travail de recherche leur fournit également des éléments clés afin de construire leur gamme et de cibler leurs produits en fonction du profil de consommation.

Mot clés: typicité, Gamay N, rotundone, vin poivré, climat froid

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INTRODUCTION

With almost 29 000 ha of vineyards in 2011 (plantgrape.plantnet-project.org), Gamay N is the eighth most planted grape cultivar in France. It is also named "*Gamay noir à jus blanc*" (black-skinned Gamay with white juice) to distinguish it from tinted Gamay cultivars (i.e. Gamay Fréaux, Gamay de Bouze and Gamay de Chaudenay), which produce grapes with colored pulp as a result of a mutation of Gamay N. Although Gamay N is thought to have first appeared in the village of Gamay near Saint-Sernin-du-Bois in Saône-et-Loire in the 1360s (Johnson, 1989), this hypothesis is unlikely because this cultivar has never been planted in the area (Robinson *et al.*, 2013). Others have suggested that Gamay N or its direct ancestors were introduced from Dalmatia by the troops of Probus, a Roman emperor (Viala and Vermorel, 1902), also improbable because genetic studies have shown that it descends from a cross between Pinot Noir N and Gouais B (Bowers *et al.*, 1999). Early to ripen and productive, Gamay N is scattered across French wine-growing regions and, according to FranceAgriMer (www.franceagrimer.fr), in 2010 represented 20 500 ha in the Burgundy and Rhône-Alpes regions (with most of the planted surface area in the Beaujolais area), 2000 ha in the Loire Valley and 1050 ha in South West France. Gamay N can also be found in several small vineyards, in cool-climate areas such as Côtes Roannaises, Côtes du Forez or Côtes d'Auvergne. Gamay N is also the second largest variety planted in Switzerland, after Pinot Noir, with about 1500 ha (Robinson *et al.*, 2013).

In most of the vineyards where it is planted, Gamay N is used to produce soft, colored, pleasant wines with fruity characteristics. These wines are made from grapes that undergo carbonic or semi-carbonic maceration (whole berries) and pre-fermentation heat treatment, except for the 10 Beaujolais crus that aim for more concentrated products and some small vineyards that cannot afford to invest in expensive heating equipment. Carbonic maceration generates higher concentrations of ethyl decanoate, eugenol, methyl and ethyl vanillates, ethyl and vinylguaiacols, ethyl and vinylphenols, ethyl cinnamate and ethyl decanoate (Versini and Tomasi, 1983 ; Ducruet, 1984 ; Fondville-Bagnol, 1996), and pre-fermentation heat treatment of grapes produces wines with higher level of ethyl esters, acetates and fatty acids (Geffroy *et al.*, 2015b). Chatelet *et al.* (2014) showed that 3-mercaptohexanol and its acetate may be involved in the blackcurrant aroma of wines made from Gamay N. However, few studies have investigated the varietal aroma of Gamay N wines.

Within the protected designation of origin (PDO) Côtes d'Auvergne, a 400-ha cool-climate vineyard located in Central France near the city of Clermont-Ferrand, Gamay N wines are known to express some unique spicy and even peppery notes. Rotundone, a sesquiterpene discovered in 2008 in an Australian Shiraz wine (Wood *et al.*, 2008), may be responsible for these distinctive flavors. This compound has been identified in an increasing number of vine cultivars, including Grüner Veltliner, Vespolina and Schioppettino in Italy (Caputi *et al.*, 2011 ; Mattivi *et al.*, 2011), Durif and Graciano in Australia (Herderich *et al.*, 2012) and Duras in South West France (Geffroy *et al.*, 2014).

Geographical influences on wine sensory profiles for a given cultivar have been studied for Sauvignon Blanc (Lund *et al.*, 2009), Malbec (Goldner and Zamora, 2007), Albariño (Vilanova and Vilarino, 2006), Touriga Nacional (Falqué *et al.*, 2004), Riesling (Douglas *et al.*, 2001), Chardonnay (Cliff and Dever, 1996 ; Schlosser et *al.*, 2005) and Pinot Noir (Cliff and Dever, 1996), but never for Gamay N. Most sensory studies highlight large differences in the sensory profiles of wines among regions of production.

To investigate the peppery typicality of Gamay N wines from the PDO Côtes d'Auvergne, a study was conducted on 21 Gamay N wines from the 2013 vintage from four French viticultural areas (Beaujolais, Loire Valley, South West and Auvergne). This research began with an assessment of the sensory differences among the wines. Standard chemical and rotundone analyses were performed in order to evaluate the variability of rotundone concentrations among the sampled wines and to correlate the peppery scores with the chemical associated with this flavor. Four wines showing distinct sensory profiles (including a peppery one) were chosen to participate in a study to assess consumer preferences. Our research work was inspired by a study conducted by Lund *et al.* (2009) on Sauvignon blanc.

MATERIALS AND METHODS

1. Wines

Vintage has a strong impact on the concentrations of grape-derived aroma compounds and those produced by yeasts, such as ethyl esters, acetates and acids, in wines made using traditional maceration techniques and/or employing pre-fermentation heat treatment of grapes (Geffroy *et al.*, 2015b). For this reason, all 21 wines in the study were chosen from the same vintage (2013). In most French wine-growing areas, the 2013 growing season was characterized by rainy conditions during the vegetative growth of the vines

and low water deficits, conditions that should be favorable to high rotundone concentrations in wines (Geffroy *et al.*, 2014). Wines (Table 1) were selected from Auvergne (Côtes d'Auvergne, including the other four red geographical indications of the PDO, i.e. Châteaugay, Madargue, Boudes and Chanturgue), South West (Gaillac), Loire Valley (Touraine, Côteaux d'Ancenis) and Beaujolais (Beaujolais Nouveau, Beaujolais, Beaujolais Villages and Brouilly). Wines were coded by a letter that refers to its region of origin (A for Auvergne, B for Beaujolais, L for Loire Valley and S for South West) and a number (from 1 to 12 for Auvergne, 1 to 6 for Beaujolais, 1 to 2 for Loire Valley and 1 for South West).

Wines were selected on the basis of being predominantly from Gamay grapes (>85%) and not being aged in oak barrels. However, one wine (A-1) was aged in oak barrels and two wines were vinified with fresh oak chips (L-1 and L-2). The retail price of the wines (which was not a criterion for selection), varied from EUR 4.50 to 9.00 VAT inclusive at the winery. With the help of contact people based in each of the four wine-growing regions, all wines were chosen for their typicality and representativeness in terms of quantity produced. The contact people also provided important information on the wines such as the winemaking techniques used, average phenology (i.e. mid-veraison date), and average climatic data for 2013 (from one weather station for Gaillac, two for the Loire Valley, three for Auvergne and seven for Beaujolais). With these climatic data, several indices endorsed by Tonietto and Carbonneau (2002) were calculated for the four regions: the Huglin index or heliothermal index from 1 April to 30 September, the cool night index (FNv-r), the mean air temperature (Tv-r), the maximal air temperature (Txv-r), and the thermal amplitude (Av-r) indices during the veraison-harvest period. Average mid-veraison dates were 25, 31, 25 and 17 August for Auvergne, Beaujolais, Loire Valley and South West, respectively. Indices were calculated on the assumption that harvest took place 45 days after mid-veraison. Cumulative rainfalls during the whole calendar year and during the budburst-veraison and veraison-harvest periods were also calculated. Wineries in Auvergne are very small and one "cuvee" is generally produced from grapes harvested on one single plot, making it possible to compile viticultural and enological data (GPS coordinates, vine spacing, altitude, picking date, use of chaptalization, temperature and length of maceration).

2. Sensory analysis

The initial expert panel was composed of 10 panelists (7 males and 3 females) who had prior experience in wine assessment. Ten training sessions were organized within 2 months prior to descriptive analysis and the panel became familiar with intensity rating of aromatic intensity ('intensity'), wine aroma sensory attributes and defects based on standard references from the Nez du Vin (Jean Lenoir, Carnoux-en-Provence, France) and the AWRI (Adelaide, Australia) as presented in Table 2 along with their corresponding odor reference standards. For in-mouth perceptions, solutions containing different concentrations of glycerol for fatness (0-20 g/L), glucose for sweetness (0-20 g/L), tartaric acid for acidity (0-1.5 g/L), quinine for bitterness (0-15 mg/L), ethanol for alcohol perception (10-15 % vol.) and alum sulfate for astringency (0-2 g/L) were presented to the panel to aid with recognition and discrimination between the different palate sensations. Tannin quality was assessed according to an unpublished, in-house-validated methodology, using three enological tannin preparations (0-1.5 g/L) provided by Oenofrance (Bordeaux, France). This methodology consists in dividing tannins into three different categories with one commercial preparation corresponding to one textural sensation on the palate: hard ("firm" tannins with good quality), green ("sticky" tannins often found in wines made from unripe grapes and that need oxygen to soften), and dry ("grainy" tannins usually extracted from oak and that cannot evolve during aging). After the training

Table 1. Number of wines included in the descriptive sensory, standard chemical and rotundone analyses (n = 21).

Region/PDO	Quantity
Auvergne	**12**
Côtes d'Auvergne	5
Côtes d'Auvergne Châteaugay	3
Côtes d'Auvergne Madargue	1
Côtes d'Auvergne Boudes	2
Côtes d'Auvergne Chanturgue	1
Beaujolais	**6**
Beaujolais Nouveau	1
Beaujolais	3
Beaujolais Villages	1
Brouilly	1
Loire Valley	**2**
Touraine	1
Côteaux d'Ancenis	1
South West	**1**
Gaillac Primeur	1

Table 2 - Aroma attributes selected for descriptive analysis and composition of the corresponding reference standards.

Attribute	Reference standard (quantities[a] or concentration)
Oxidation level	1 drop each of sample 6 (sulfur) and 9 (cauliflower) of Le Nez du Vin "*Les défauts*" for reduced aroma (0 on the rating scale)
	1 drop of sample 2 (overripe apple) of Le Nez du Vin "*Les défauts*" for oxidized aroma (5 on the rating scale)
Defect	1 drop of sample 3 (vinegar) of Le Nez du Vin "*Les défauts*" for acetic acid bacteria spoilage
	1 drop of sample 12 (cork) of Le Nez du Vin "*Les défauts*" for cork taint
	1 drop of sample 10 (horse) of Le Nez du Vin "*Les défauts*" for *Brettanomyces* contamination
Fermentative/amylic	1 drop of sample 5 (banana) of Le Nez du Vin
Fermentative/lactic	10 mL of liquid cream, Délisse
Floral	1 drop of sample 29 (violet) of Le Nez du Vin
Thiol	1 drop each of sample 2 (grapefruit) and 37 (blackcurrant bud) of Le Nez du Vin
Terpenic	1 drop each of sample 12 (strawberry) and 13 (raspberry) of Le Nez du Vin
Spicy/peppery	200 ng/L of rotundone in neutral red wine
Spicy/licorice	1 drop of sample 36 (licorice) of Le Nez du Vin
Green	1 drop of sample 30 (green bell pepper) of Le Nez du Vin
Oaky	1 drop each of sample 48 (toast) and 49 (roasted almonds) of Le Nez du Vin

[a]Quantities specified are those added to 40 mL of neutral red wine.

sessions, two panelists (one male and one female) were excluded from the final panel because they could not detect rotundone and peppery flavors. Specific anosmia to this compound has been reported : for example, approximately 20 % of panelists cannot detect this compound in sensory trials at very high concentrations (4000 ng/L), even in water (Wood *et al.*, 2008). The wine sensory descriptive analysis was performed in accordance with the ISO 11035 (1994) standard in a professional room. A constant volume of 10 mL of each wine was poured in black wine-tasting glasses at 15 °C. Each panelist evaluated each of the 21 wines on 30 June 2014 once in randomized order to rate the intensity of sensory attributes. The intensity of each attribute was scored on a five-point rating scale, on which "0" indicated that the attribute was not perceived and "5" that it was perceived at very high intensity (except for oxidation level whose scale ranged from zero - having a reduced aroma - to five - having an oxidized aroma). For defect, the panelists did not have to describe the nature of the defect but only to rate its intensity if attributes associated with acetic acid bacteria spoilage, *Brettanomyces* contamination or cork taint were perceived. Presentation order of the 21 samples, coded with three-digit codes and served blind, was randomized for each panelist. Both presentation order and number codes were determined using Tastel software (ABT Informatique, France).

3. Standard chemical and rotundone analyses

Conventional enological parameters and rotundone levels were determined for the 21 bottled wines in mid-July 2014, less than one month after the descriptive analysis and consumer study. Alcohol content was measured using an Alcoquick L200 infrared analyzer (Unisensor, Germany) and pH with a Titromatic pH meter (Hachlange, Germany). Total acidity was measured according to the OIV method (OIV, 2009). A Konelab Arena 20 sequential analyzer (Thermo Electron Corporation, USA) was used with enzyme kits to determine volatile acidity (Megazyme, Ireland) and glucose/fructose (Thermo Fisher Scientific, USA). Anthocyanins and total phenolic index (TPI) were quantified according to the techniques described in Ribéreau-Gayon and Stonestreet (1965) and Ribéreau-Gayon (1970), respectively, using an Evolution 100 spectrophotometer (Thermo Electron Corporation, USA). All determinations were carried out in duplicate. Rotundone concentrations in wine were determined by the AWRI, as part of a contract service, using solid phase microextraction-multidimensional gas chromatography-mass spectrometry (Geffroy *et al.*, 2014).

4. Consumer study

Panelists, all of whom were self-declared red wine consumers, were recruited from wine shops, by word of mouth, and, for the Parisian session, via social networks and announcements in leading French wine magazines (*La Revue des Vins de France*, *Le Figaro Vin*). Remuneration for participating in the study consisted of a bottle of wine and a book. Consumers knew only they were tasting some red wines. They provided demographic information and responded to purchase behavior questions. Socio-economic classification was determined according to French National Institute for Statistics and Economics

(INSEE) categories: farmers, mid-level occupations, managers and professionals, service, sales and support workers, craft and trades workers, elementary occupations, students, and unemployed workers. Two distinct sessions were organized on 1 July and 2 July 2014 in Auvergne, in the city of Clermont-Ferrand (n = 47), and in Paris (n = 40), respectively. The 87 consumers evaluated all four wines, chosen from the 21 included in the sensory study for their distinct sensory profile. The consumer evaluation was not organized in a professional tasting room with booths but in a neutral room with white walls. Consumers were sufficiently apart from each other to ensure that no communication occurred. Samples were anonymous and presentation order was randomized. A constant volume of 10 mL of each wine was poured in transparent wine-tasting glasses at 15 °C. Ranking tests were performed and the consumer panelists first had to carry out an olfactory assessment based on aroma preferences, and then a taste assessment based on flavor and texture preferences (i.e. acidity, balance, bitterness, quantity and quality of tannins). Wines were ranked by the panelists from 1 ("the favorite") to 4 ("the least appreciated"). The panelists were allowed to retaste samples if requested.

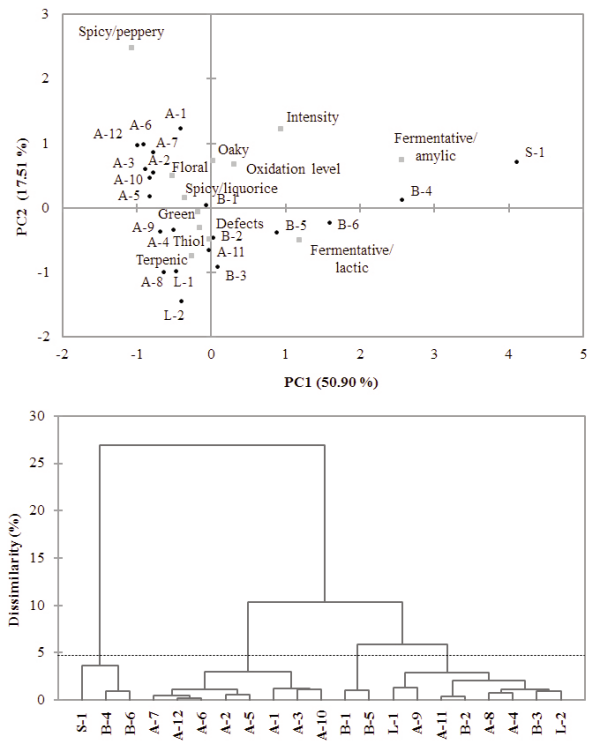

Figure 1 - (A) Principal component analysis (PC1 vs PC2) and (B) agglomerative hierarchical clustering of olfactory data of 21 Gamay N wines from four French wine-growing areas. A: Auvergne (n = 12); B: Beaujolais (n = 6); L: Loire Valley (n = 2); S: South West (n = 1).

5. Statistical treatment

Statistical analyses including linear regressions were conducted using Xlstat software (Addinsoft, France). For each of the 21 wines, olfactory data from the descriptive analysis were first averaged over the panelists, treated with principal component analysis (PCA). In order to select the four wines used in the consumer study, an agglomerative hierarchical clustering (AHC) was performed directly on the 12-olfactory descriptor matrix. Then, a one-way analysis of variance (ANOVA) in which region was the factor and panelists and wines were considered as repetitions was carried out on the data from the sensory analysis. Chemical data were also treated through ANOVA considering the region as a factor and wines as repetitions. For the four wines selected for the consumer study, olfactory and taste data were tested in a one-way analysis of variance (ANOVA). Fisher's least significant difference test was used as a post-hoc comparison of means at $P \leq 0.05$. Consumer data were analyzed using Friedman's test followed by a Nemenyi post-hoc test at $P \leq 0.05$.

RESULTS AND DISCUSSION

1. Sensory analysis

The PCA plot (Figure 1A) shows that four attributes explain the main differences observed between the 21 Gamay N wines of the study: intensity, fermentative/lactic, fermentative/amylic and spicy/peppery. The scores discriminated a group of wines from Auvergne with similar sensory characteristics (A-1, A-2, A-3, A-5, A-6, A-7, A-10 and A-12) and heavier peppery notes. Results of the AHC (Figure 1B) led to the same conclusions. S-1, B-4 and B-6 wines had pronounced fermentative/amylic notes, and B-1 and B-5 showed a fermentative/lactic aroma sensory profile. These fermentative aromatic features are likely related to the specific winemaking techniques used. Several studies, in particular that of Geffroy *et al.* (2015b), have shown that pre-fermentative heat treatment of grapes induces a significant increase in acetates, fatty acids and most ethyl esters when wines are fermented in the liquid phase. The other wines of the study showed less distinctive aroma profiles. Grouped by region of production, wines from Auvergne had more intense peppery characteristics (Table 3) with large variability for this attribute. Wines from the Loire Valley were less expressive and had lower intensity. Produced in the northern-most vineyards of this study, these wines were perceived by the panelists as thinner, more acidic, more reductive and with less sweetness than the other wines of the study. Wines from the Beaujolais and the South West of France showed a quite similar

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Table 3 - Sensory attributes of the Gamay N wines rated on a five-point scale and grouped by region of production. Data were averaged over the panelists and over the wines from one region. Standard deviations (SD) refer both to within-panelist and within-wine variabilities with the exception of the South West for which it only refers to within-panelist variability.

Sensory attribute	*P*-value	Auvergne (n = 12)		Beaujolais (n = 6)		Loire Valley (n = 2)		South West (n = 1)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
Olfactory									
Intensity	< 0.0001	3.30 b[a]	0.91	3.40 b	0.86	2.60 c	0.84	4.80 a	0.44
Oxidation level	0.045	2.63 a	0.88	2.77 a	0.57	2.00 b	0.94	3.00 a	0.00
Defect	0.349	0.22 a	0.65	0.03 a	0.18	0.30 a	0.95	0.00 a	0.00
Fermentative/amylic	< 0.0001	0.08 c	0.42	0.93 b	1.44	0.00 c	0.00	3.80 a	0.81
Fermentative/lactic	< 0.0001	0.05 b	0.39	1.13 a	1.46	0.40 b	1.27	1.80 a	1.12
Floral	0.060	0.75 a	1.16	0.30 a	0.88	0.10 a	0.32	0.00 a	0.00
Thiol	0.360	0.15 a	0.52	0.37 a	0.81	0.30 a	0.67	0.00 a	0.00
Terpenic	0.640	0.85 a	1.05	1.07 a	0.94	1.20 a	1.03	0.80 a	0.63
Spicy/peppery	< 0.0001	1.53 a	1.51	0.53 b	1.12	0.10 b	0.32	0.00 b	0.00
Spicy/licorice	0.383	0.65 a	1.02	0.33 a	0.66	0.60 a	1.07	0.20 a	0.45
Green	0.207	0.20 a	0.68	0.00 a	0.00	0.40 a	0.84	0.00 a	0.00
Oaky	0.715	0.48 a	1.04	0.33 a	0.66	0.20 a	0.42	0.00 a	0.89
Taste									
Fatness	< 0.01	1.98 a	0.91	2.17 a	0.87	1.10 b	0.99	2.60 a	1.14
Sweetness	0.024	0.48 ab	0.79	1.03 a	1.35	0.30 b	0.67	1.40 a	1.01
Acidity	0.026	1.20 b	1.22	0.80 b	1.06	2.10 a	1.19	0.60 b	1.14
Astringency	0.478	2.10 a	0.97	2.37 a	0.89	1.90 a	0.88	2.20 a	0.73
Bitterness	0.707	0.12 a	0.52	0.03 a	0.18	0.20 a	0.63	0.20 a	0.34
Alcohol perception	0.609	1.20 a	1.29	1.03 a	1.19	0.80 a	1.13	0.60 a	1.10
Hard tannins	0.109	1.55 a	1.11	1.87 a	0.97	0.90 a	1.10	0.60 a	1.01
Green tannins	0.846	0.53 a	0.83	0.70 a	1.02	0.70 a	0.67	0.60 a	0.74
Dry tannins	0.150	0.23 a	0.65	0.00 a	0,00	0.40 a	0.66	0.16 a	0.37

[a]Means with the same letter within a row are not significantly different according to the least significant difference test at $P \leq 0.05$.

sensory profile with more intense fermentative/lactic and fermentative/amylic notes. For this last attribute, the wine from the South West, which was the most intense and rather aromatically simple, received the highest score.

2. Standard chemical and rotundone analyses

Together with the wine from the South West, wines from Beaujolais were characterized by higher pH associated with lower acidity (Table 4). In accordance with the sensory observations, wines from the Loire Valley showed the lowest pH. Among the wines from Auvergne, large variability was observed for most studied chemical attributes (i.e. alcohol content, total acidity). The 2013 vintage was late in ripening due to rainy weather conditions in early October in Auvergne. Therefore, the observed variability might reflect differences in behavior among winegrowers : those who chose to harvest grapes early to limit *Botrytis cinerea* severity and those who took the risk to wait longer until better ripening. For rotundone (Figure 2), there were no differences between the wines from Beaujolais, Loire Valley and South West. The Gamay N wines from Auvergne had higher concentrations in this aroma compound, corroborating the results of the descriptive analysis. Among the 12 wines from this region, four had a rotundone content greater than 100 ng/L, with a maximum of 142 ng/L measured in wine A-7. The 12 Gamay N wines from Auvergne were produced using traditional winemaking techniques with maceration durations ranging from 5 to 31 days, whereas the other wines in the study all contained a percentage of wine produced by semi-carbonic (whole berries) maceration or pre-fermentation heat treatment of grapes. As rotundone is a hydrophobic compound that is extracted from the berries during the first days of alcoholic fermentation by the solvent effect of ethanol (Siebert and Solomon, 2011), semi-carbonic maceration and pre-fermentation heat treatment do not *a priori* promote

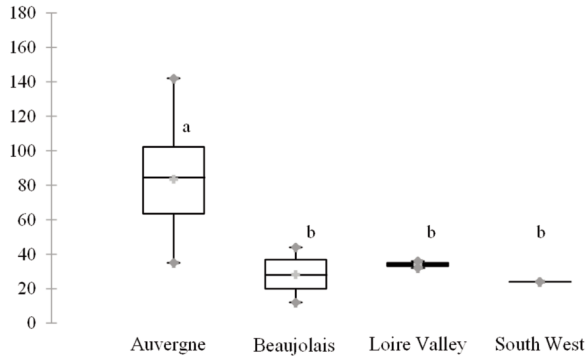

Figure 2 - Rotundone concentrations in Gamay N wines according to region of production.
Different letters indicate means significantly different at $P \leq 0.05$ by Fisher test.

the production of wines with high rotundone concentrations. Therefore, it remains to be determined if the higher levels found in the wines from Auvergne reflect a real "terroir" effect or arise from specific winemaking techniques. Several hypotheses in regard to climate conditions have been proposed to explain the variation in rotundone levels among vintages, vineyards or within the same plot. According to Caputi *et al.* (2011), the cool and rainy vintages or vineyards are particularly favorable to the accumulation of rotundone in grapes. According to Geffroy *et al.* (2014), water status experienced by the vine late in the growing season is a key variable in inter-vintage variation in rotundone. More recently, Zhang *et al.* (2015) showed that berry temperature exceeding 25 °C during the veraison-to-harvest period negatively affects the rotundone concentration in Shiraz and that temperature was one of the main determinants of rotundone in grape berries. Climatic indices presented in Table 5 help compare the climate in the four viticultural areas accurately. Auvergne is 1) the coolest vineyard over the whole wine-growing season and the ripening period, and 2) the wettest during the veraison-harvest period, although it is the driest over the whole calendar year. Its "terroir" via its climate component is therefore the most likely factor explaining the differences observed between the wines from Auvergne and the other regions of the study. These differences may be further amplified by the winemaking techniques.

3. Relationship between rotundone and spicy/peppery aroma

Wine is a complex matrix containing hundreds of volatiles that can act in synergy, and in which ethanol and aroma compounds can operate as a buffer or as a mask (Ferreira, 2012). Despite the complexity of wine aroma and considering the expected high concentrations in esters and acetates in wines made using alternative winemaking techniques, a significant correlation (Figure 3) was observed between peppery aroma scores and rotundone concentration in wine ($r^2 = 0.66$). This conclusion corroborates observations made by Ferreira (2012), who described rotundone as one of the 16 most "impacting" aroma compounds in wine. The observed coefficient of determination is comparable to values obtained in previous studies investigating the link between chemical compounds and sensory data. Coefficients of determination ranging from 0.50 to 0.80 have been reported, for example, in white wines made from Albariño (Vilanova *et al.*, 2010), Sauvignon blanc (Lund *et al.*, 2009) and in red wines from Cabernet-Sauvignon and Merlot (Roujou de Boubée, 2000).

4. Variability in rotundone among the samples from Auvergne

There was high variability in rotundone content among the 12 wines from Auvergne, with the lowest value of 35 ng/L measured in wine A-8, and the highest value reaching 142 ng/L in wine A-7. As the 12 wines from Auvergne were each made from a

Table 4 - Conventional enological parameters of the Gamay N wines according to region of production.

Parameter	*P*-value	Auvergne (n = 12)		Beaujolais (n = 6)		Loire Valley (n= 2)		South West (n = 1)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
Alcohol content (% vol.)	0.322	12.35 a[a]	0.69	12.30 a	0.18	11.68 a	0.46	11.58 a	-
Total acidity (g/L H_2SO_4)	< 0.001	3.96 a	0.27	3.38 b	0.22	4.00 a	0.04	2.82 b	-
pH	< 0.0001	3.48 c	0.08	3.59 b	0.11	3.21 d	0.02	3.85 a	-
Volatile acidity (g/L acetic acid)	0.031	0.43 a	0.13	0.28 b	0.06	0.25 b	0.01	0.21 b	-
Glucose/Fructose (g/L)	0.691	0.64 a	0.91	0.35 a	0.52	0.20 a	0.30	0.00 a	-
Total phenolic index	0.317	51 a	9	55 a	10	41 a	1	52 a	-
Anthocyanins (mg/L)	0.268	234 a	81	307 a	61	232 a	1	264 a	-

[a]Means with the same letter within a row are not significantly different according to the least significant difference test at $P \leq 0.05$.

Table 5 - Characterization of the 2013 vintage in the four regions of the study according to several climatic indices and cumulative rainfall calculated over the whole calendar year and the budburst-veraison and veraison-harvest periods.

Region	Huglin index (IH)	Cool night index (FNv-r)	Mean air temperature (Tv-r)	Maximal air temperature (Txv-r)	Thermal amplitude (Av-r)	Cumulative rainfalls (mm)		
						01/01 - 31/12	Budburst - veraison	Veraison - harvest
Auvergne	1490	11.4	15.2	20.8	9.4	599	366	105
Beaujolais	1731	11.8	15.5	21.0	9.2	801	419	88
Loire Valley	1574	12.4	16.8	22.1	9.7	722	232	57
South West	1909	12.3	17.8	24.7	12.4	782	347	48

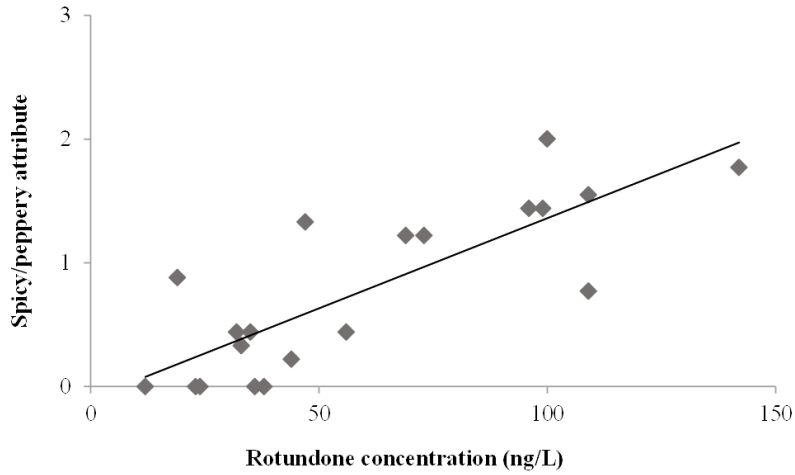

Figure 3 - Relationship between rotundone concentration in wines and their spicy/peppery score rated on a five-point scale (n = 21). Linear regression model: y = 0.0146x – 0.0969; *P* < 0.0001; r^2 = 0.66.

single vineyard plot, we mapped wine rotundone concentrations according to the plots from which the grapes were primarily sourced (Figure 5). The rotundone distribution does not appear to be spatially structured. Although soil characteristics and climatic data likely play a role, "terroir" may not be the dominant factor explaining the differences observed. Based on previous studies on rotundone, the observed variability may have a multifactorial explanation involving parameters related to plant material (Siebert and Solomon, 2011; Geffroy *et al.*, 2015a), level of ripening, water deficit and wine-growing techniques such as leaf removal (Geffroy *et al.*, 2014) and winemaking techniques. Several viticultural and enological data were collected for the 12 plots from Auvergne. One wine from Auvergne (A-4) was chaptalized, which led to an increase in ethanol concentration in the final wine (+1 % vol.). The only parameter that accounted for the variability among the wines was alcohol content. After removing from the treatment the data associated with the chaptalized wine, there was a significantly positive correlation at $P < 0.05$ (Figure 4) between this alcohol content and rotundone ($r^2 = 0.44$). On the one hand, this result confirms previous studies by Geffroy *et al.* (2014) and Caputi *et al.* (2011), which have shown that rotundone concentration increases with the level of ripening. On the other hand, higher ethanol concentrations may have induced higher extraction of rotundone from the skin during the alcoholic fermentation. However, the effect of the differences in ethanol should be minimal because studies have shown that the impact of ethanol on the extraction of hydrophobic compounds, such as proanthocyanidins, do not exceed a few percent (Canals *et al.*, 2005).

5. Wines selected for the consumer study and composition of the panel

The sensory characteristics of the four wines selected for the consumer study (S-1, B-1, A-6 and A-8) are shown in Table 6. S-1 whose sensory profile can be qualified as 'amylic', had higher aromatic intensity and heavy fermentative/amylic and fermentative/lactic notes. B-1 had a more complex profile marked by significantly higher 'lactic' notes. A-6 and A-8 were characterized by 'peppery' and 'green' notes,

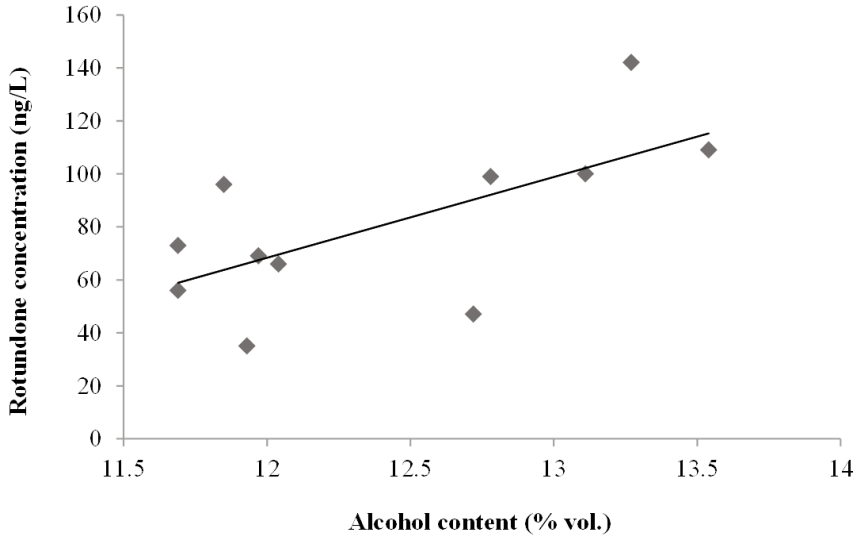

Figure 4 - Relationship between alcohol content and rotundone concentration in wines from Auvergne (n = 11). One data point from a chaptalized wine was removed from the treatment. Linear regression model: y = 30.49x -298; $P < 0.05$; $r^2 = 0.44$.

Table 6 - Sensory attribute scores of the four wines chosen for the consumer study based on a five-point rating scale and rotundone concentration (n = 8).

Attribute	*P*-value	S-1		B-1		A-6		A-8	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
Olfactory									
Intensity	0.004	4.43 a[a]	0.82	2.86 b	1.17	3.29 b	0.75	2.57 b	1.05
Oxidation level	0.644	3.00 a	0.00	2.80 a	0.50	2.60 a	0.55	2.00 a	0.96
Defect	0.130	0.00 a	0.00	0.00 a	0.00	0.00 a	0.00	0.43 a	0.00
Fermentative/amylic	< 0.001	3.43 a	1.94	0.14 b	0.00	0.00 b	0.00	0.00 b	0.00
Fermentative/lactic	0.016	1.14 a	1.51	1.43 a	1.21	0.00 b	0.00	0.00 b	0.00
Floral	0.224	0.00 a	0.00	0.57 a	1.03	0.00 a	0.00	0.71 a	0.82
Thiol	0.649	0.00 a	0.00	0.29 a	0.51	0.00 a	0.00	0.14 a	0.41
Terpenic	0.924	0.43 a	0.55	0.80 a	0.98	0.57 a	0.82	0.57 a	0.52
Spicy/peppery	< 0.001	0.00 b	0.00	0.86 b	1.03	2.57 a	1.37	0.43 b	0.52
Spicy/licorice	0.801	0.14 a	0.41	0.00 a	1.03	0.14 a	0.41	0.14 a	0.41
Green	0.039	0.00 b	0.00	0.00 b	0.00	0.00 b	0.00	1.14 a	1.25
Oaky	0.791	0.43 a	0.41	0.14 a	0.00	0.43 a	0.41	0.14 a	0.00
Taste									
Fatness	0.635	2.29 a	1.17	2.29 a	1.05	2.29 a	0.82	1.71 a	1.03
Sweetness	0.210	1.00 a	1.47	0.57 a	0.84	0.14 a	0.41	0.14 a	0.00
Acidity	0.795	1.14 a	1.51	1.14 a	1.03	1.14 a	1.51	1.71 a	1.21
Astringency	0.049	1.71 a	0.71	1.86 a	0.71	2.00 a	0.73	0.71 b	0.75
Bitterness	0.542	0.14 a	0.41	0.00 a	0.00	0.00 a	0.00	0.29 a	0.82
Alcohol perception	0.770	0.43 a	0.00	1.00 a	1.03	0.86 a	1.26	1.00 a	0.98
Hard tannins	0.098	1.23 a	1.17	1.86 a	1.17	1.43 a	0.98	0.43 a	0.84
Green tannins	0.840	0.86 a	1.55	0.71 a	0.82	0.43 a	0.52	0.71 a	0.41
Dry tannins	0.152	0.00 a	0.00	0.00 a	0.00	1.00 a	0.98	0.28 a	0.84
Rotundone (ng/L)	-	24	-	38	-	100	-	35	-

[a]Means with the same letter within a row are not significantly different according to the least significant difference test at $P \leq 0.05$.

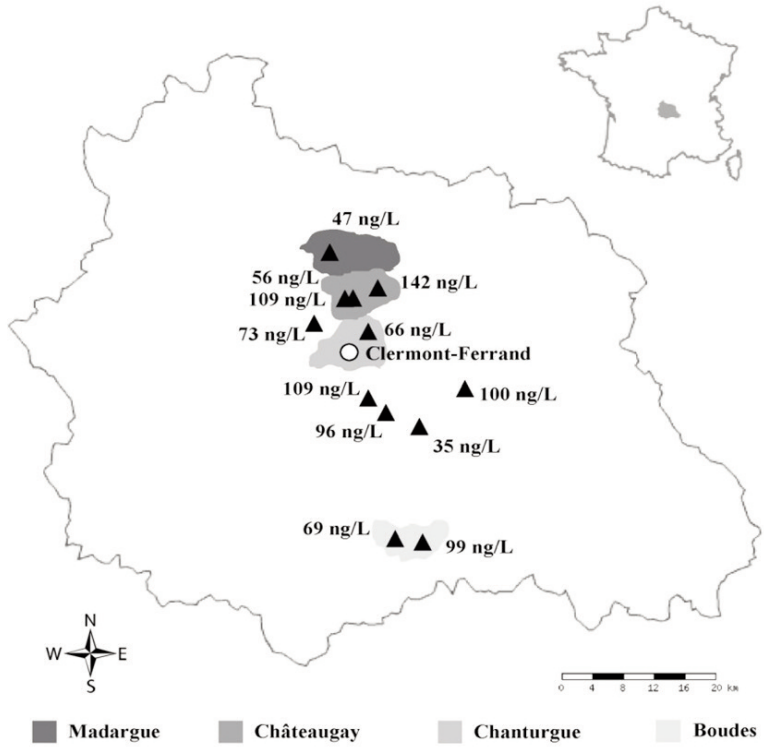

Figure 5 - Map of the grape sources for the 12 wines from Auvergne and the rotundone concentrations in those wines. The PDO areas of the four red wine geographical indications (Madargue, Châteaugay, Chanturgue and Boudes) are also shown.

respectively. A-8 had the lowest astringency level among the four wines.

6. Consumer preferences

Demographic information from the Clermont-Ferrand and Paris wine consumers is given in Table 7. Marked differences in terms of panel composition and consumption habits were observed between the two cities. The Parisian panel was younger, included more women, and was mainly composed of managers and professionals. Panelists in Paris had more regular wine consumption habits with 78 % of them drinking wine 3 to 4 times a week, and spend more money on wine in comparison with the consumers from Clermont-Ferrand. Pooled together, the two panels showed a globally well balanced age and gender distribution. Results given in Table 8 show that there were few differences in terms of consumer preferences between the two cities. We therefore pooled the two panels. Regarding the olfactory assessment of the wines, there were no significant differences according to Friedman's test between the four wines. A lack of consensus was observed for wine S-1 as it was rated 37 and 27 times as the favorite and the least appreciated, respectively. The group who judged this wine as the favorite was mainly composed of consumers older than 45 years and contained a larger proportion of females. In the same way, no general agreement was observed for wine A-6, and consumers who rated the 'peppery' wine as the most appreciated were managers and professionals who are willing to pay more for a bottle of wine. It is important to notice that a large percentage of consumers who preferred A-6 was between 35 and 45 years old. The group of consumers who preferred the 'amylic' wine frequently rated the 'peppery' wine as the least appreciated and vice versa. This led us to think that there is an opposition between the consumers who preferred the 'amylic' and the 'peppery' wines. Due to the specific anosmia reported for rotundone, it would have been interesting to investigate whether consumers who preferred or did not appreciate wine A-6 were able to perceive its peppery notes. However, this type of investigation would require a specific tasting session using water solutions spiked with rotundone at several concentrations. Wines A-8 and B-1 were less frequently cited as the favorite and/or least appreciated, being rated 34 times out of 87 as the second and third favorite wines, respectively, thus eliciting a more middle-of-the-road judgment. Those who preferred these two wines were mainly consumers younger than 35 years old. Differences were significant and more marked for taste evaluation, and A-8, which presents the lowest

Table 7 - Demographic information on the Clermont-Ferrand (n = 47) and Paris (n = 40) panelists.

Demographic information		Clermont-Ferrand	Paris	Pooled panels
Gender	Male	77%	55%	67%
	Female	23%	45%	33%
Age (years)	18-24	2%	15%	8%
	25-34	6%	45%	24%
	35-44	19%	20%	20%
	45-54	32%	10%	22%
	>55	41%	10%	26%
Socio-economic classification[a]	Farmers	4%	0%	2%
	Middle-level occupations	18%	3%	10%
	Managers and professionals	49%	72%	62%
	Service, sales and support workers	16%	2%	9%
	Craft and trades workers	11%	15%	13%
	Elementary occupations	2%	0%	1%
	Students	0%	8%	3%
	Unemployed workers	0%	0%	0%
Wine preference	Red	89%	90%	90%
	White	11%	10%	10%
	Rosé	0%	0%	0%
Wine consumption (frequency)	Once a day	24%	5%	15%
	3-4 times a week	39%	78%	57%
	Once a week	28%	17%	23%
	Twice a month	9%	0%	5%
	Once a month	0%	0%	0%
Average price spent on bottle	<€3	0%	0%	0%
	€3 to €5	21%	2%	12%
	€5 to €10	49%	35%	42%
	>€10	30%	63%	46%

astringency level, was the only wine listed as favorite.

CONCLUSION

This study was conducted on 21 Gamay N wines from four French viticultural areas to better comprehend the aromatic typicality of Gamay N wines from cool-climate vineyards (PDO Côtes d'Auvergne). In comparison with the other regions in the study, wines from Auvergne were characterized by more intense peppery notes with higher rotundone concentrations. These sensory features are likely to be due to a "terroir" effect with Auvergne being the coolest vineyard over the whole wine-growing season and the ripening period, and the wettest during the veraison-harvest period. There was a significantly positive correlation ($r^2 = 0.66$) between spicy/peppery notes and analyzed rotundone levels. Within the PDO Côtes d'Auvergne, there was high variability in rotundone concentrations, which was attributed to the alcohol content of the wines, a factor that was significantly positively correlated with rotundone ($r^2 = 0.44$). Consumer studies performed in two cities (Paris and Clermont-Ferrand) on four wines showing distinct sensory profiles ('amylic', 'lactic', 'peppery' and 'green'), provided interesting conclusions. While no significant differences were observed between the wines for the olfactory assessment, A-8 – the wine with the lowest astringency level – was the only wine of the four preferred in the taste assessment. There was an opposition between the consumers who preferred the 'amylic' and the 'peppery' wines. Those who appreciate wines with a peppery sensory profile were generally managers and professionals who are willing to pay more for a bottle of wine. From a commercial and sales development point of view, these results that scientifically investigated the typicality and originality of Gamay N wines from Auvergne should assist winegrowers from this region in promoting their wines. They allow them to take advantage of the knowledge on rotundone obtained in another wine-growing regions with the aim to produce wines with a desired level of peppery aroma. The consumer study provides the key parameters for developing the Côtes d'Auvergne Gamay N wine range and adapting the products to consumer profiles.

Table 8 - Results of the two consumer studies held in Clermont-Ferrand (n = 47) and Paris (n = 40). Wines were ranked by the panelists from 1 « the favorite » to 4 « the least appreciated ».

Panel	Results	Wine / sensory profile			
		S-1 'Amylic'	B-1 'Lactic '	A-6 'Peppery'	A-8 'Green'
Clermont-Ferrand	**Olfactory evaluation**				
	Sum of the ranks	104 a[a]	130 a	129 a	107 a
	Taste evaluation				
	Sum of the ranks	118 ab	125 ab	132 b	95 a
Paris	**Olfactory evaluation**				
	Sum of the ranks	97 a	105 a	105 a	92 a
	Taste evaluation				
	Sum of the ranks	111 a	102 a	101 a	86 a
Both panels (pooled)	**Olfactory evaluation**				
	Sum of the ranks	201 a	235 a	234 a	199 a
	Number of times rated as the favorite	37	8	21	21
	Number of times rated as the least appreciated	27	19	27	14
	% of consumers who are female when favorite	38	12	29	33
	% of consumers who are managers and professionals when favorite	53	62	67	60
	% of consumers who are older than 45 years when favorite	59	13	43	38
	% of consumers who are between 35 and 45 years old when favorite	20	37	33	10
	% of consumers who are younger than 35 years when favorite	21	50	24	52
	% of consumers who pay more than €5 when favorite	84	75	90	76
	% of consumers who pay more than €10 when favorite	38	25	62	43
	Number of times rated as the least appreciated when A-6 (peppery) is rated as the favorite	12	5	-	4
	Number of times rated as the least appreciated when S-1 (amylic) is rated as the favorite	-	9	19	9
	Taste evaluation				
	Sum of the ranks	229 b	227 b	233 b	181 a

[a]Different letters within the same row indicates sums of the ranks significantly different according to the Nemenyi test at $P \leq 0.05$.

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