Recent Amendment to Product Specification of Brisighella PDO (Emilia-Romagna, Italy): Focus on Phenolic Compounds and Sensory Aspects

Recent amendment to product specification of Brisighella PDO (Emilia Romagna, Italy): focus on phenolic compounds and sensory aspects

Sara Barbieri, Alessandra Bendini*, Tullia Gallina Toschi.

Department of Agricultural and Food Sciences (DiSTAL), University of Bologna, Piazza Goidanich 60, 47521 Cesena (FC), Italy

*Correspondence: Alessandra Bendini, Department of Agricultural and Food Sciences (DiSTAL), University of Bologna, Piazza Goidanich 60, 47521 Cesena (FC), Italy. Phone: 139 0547 338121, e-mail: alessandra.bendini@unibo.it

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List of abbreviations:

DO, Designation of Origin; EVOO, Extra Virgin Olive Oil; FA, Free Acidity; FAME, Fatty Acid Methyl Esters; IOC, International Oil Council; PDO, Protected Designation of Origin; PGI, Protected Geographical Indication; PV, Peroxide Value; TSG, Traditional Speciality Guaranteed; VOO, Virgin Olive Oil.

Abstract

The Brisighella Protected Designation of Origin (PDO) extra virgin olive oil (EVOO) has been protected by European trademark since 1996. It is obtained from olives belonging to the cv "Nostrana di Brisighella" (must make up at least 90% of groves) grown exclusively in Brisighella, a circumscribed area of Emilia-Romagna region, in the north-central of Italy. Brisighella PDO EVOO is produced by the unique plant mill of the Consortium (Consorzio Olio DOP “Brisighella”).

In this research, minor amendments to product specification of Brisighella PDO approved by EU in 2016 are described. The proposed modifications to the Brisighella PDO product specification are supported by chemical-sensory analyses carried out on 15 EVOOs from cultivars autochthonous of the Emilia Romagna region (Nostrana di Brisighella and Ghiacciolo), selected as representative of 14 geographical sites (small farms) with different characteristics in terms of climatic and environmental conditions (e.g. altitude, geographical orientation and slope of the olive orchard) of the production area established in the product specification. These results were also compared with available data of EVOOs certified as POD Brisighella in the period 2004-2012 and commissioned by the responsible certification control body.

Pratical application:

The product specification represents the product’s identification document that must contain a description of the requirements that the product must comply with (e.g. product description in terms of chemical and sensory characteristics, delimitation of geographical area and evidence of the link with the territory, methodology applied etc.). It also must include the references relative to control systems; therefore, amendments according to the updating of EU legislation in the sector are desirable to assure the final consumer that the expected value of the product effectively corresponds with what has been declared. Moreover, considering the growing interest of consumers towards health properties, this update will contribute to communicate the intrinsic properties of olive oil and benefits to health, enhancing the product image and promoting informed consumption.

Introduction

Protected designations of origin (PDO) represent food products that possess specific qualities with respect to other ones in the same category due to an extremely strong link to the territory. Protection tools for this product category have been established in all European Union countries to valorise designations of origin.

European quality products (also including Protected Geographical Indication, PGI and Traditional Speciality Guaranteed, TSG) must comply with product specifications/regulations and are subject to strict controls by third party bodies which make them easily recognisable and distinguishable from other conventional products for consumers [1-3]. Benefits from the protection given by EU for designation of origin (DO) not only represent the quality of products, but also constitute a guarantee for their authenticity.

Mediterranean countries are the central core of European geographical indications (PDO, PGI and TSG), with Italy, France, Spain, Greece and Portugal alone having 71% of food DO products. Italy has the highest number of geographical indications in the food and wine sectors (over 800 as of 2017) and Emilia Romagna is the region with the highest number of food registered products [4].

Concerning the category of olive oils, Italy reaches a total of 46 certified products (42 PDO and 4 PGI) (http://ec.europa.eu/agriculture/quality/door/list.html). Despite the widespread presence of DO in all olive-growing areas in Italy, certified products represent just over 2% of the total amount produced by the sector [4].

One of the first designations of origin registered in Italy for EVOO was Brisighella PDO which dates back 1996. Since then, numerous amendments to EU regulations have taken place, and above all in relation to sensory analysis. A method for organoleptic evaluation of olive oils was introduced in 1991 [5] by a regulation inspired by the IOC/T.20/Doc. no.3, published in 1987 [6]. This first version of the method was adopted and included in Brisighella PDO product specification [7].

The International Olive Oil Council (IOC) subsequently modified this method to make it simpler and more reliable [8]. The main modifications were made in 2002 and concerned: i) reduction of the

sensory attributes compared to the old profile sheet (only sensory defects and the three most important positive attributes: fruity, pungent and bitter); ii) adoption of continuous scales (from 0 to 10 cm) for evaluating the intensity of perception of the different attributes; iii) adoption of a statistical elaboration method for classifying oils according to the median of the defect perceived with greatest intensity and the median for fruity [9]. In 2008, a new upper limit of the main perceived defect was introduced for discriminating between virgin and lampante olive oils (from 2.5 to 3.5) and grouping in two different defects in only one negative attribute: fusty and muddy sediment [10].

In 2005, the IOC issued a document on the methods to be used for organoleptic assessment of EVOO for granting DO status [11]. This document declared that the DO authority shall select the characteristic descriptors of the designation of origin (up to 10) from those defined and incorporate them into the profile sheet of the method. The characteristic descriptors are identified according to the round-table method using a series of samples representing the most important specific characteristics of the DO. Most of the specifications for the DO of oils before 2005 or those that have not undergone revisions after this date (e.g. Brisighella PDO) do not refer to the method IOC just explained, but to the use of a previous procedure [5] for sensory evaluation of oils.

It is well known that the sensory quality of virgin olive oils (VOOs) is mainly due to the presence of minor compounds, such as volatile and phenolic molecules [8]; nevertheless, the evaluation of profiles in these minor compounds are not yet recognised among the official chemical parameters provided by European Regulations on assessment of the quality of VOO.

In 2009, the IOC approved an official method [12] for determination of biophenols in olive oils by HPLC. As reported by several authors, even in small quantities, phenols are fundamental for sensory attributes (bitter and pungent), for the stability of oil during the storage (antioxidant activity) and for its nutraceutical effects [13-14].

In 2012, the European Food Safety Authority (EFSA) provided a scientific opinion on a health claim related to polyphenols in olive oil and the maintenance of normal blood HDL-cholesterol concentrations establishing that the health claim for olive oil polyphenols can be added when the

content (bound and free forms of hydroxytyrosol and tyrosol) is at least 5 mg for 20 g of oil and by specifying that the health benefits are achieved with a daily intake of 20 g of olive oil having this minimum content [15].

Herein, the main amendments to the Brisighella PDO product specification are described. All modifications followed the update of EU legislation in the sector, adopted subsequently to the registration of Brisighella PDO and related characteristics that are proper to EVOOs in general and not specific to Brisighella PDO, have been approved as minor according to EU Regulation No 1151/2012. Specifically, the main modifications concern: i) replacement of the date of harvest with the indication to consider the progression of seasons and the start of ripening; ii) updating chemical-sensory parameters; iii) the possibility of indicating guarantees on the label to the product's health claim related to biophenol content, as Brisighella PDO has the characteristics to warrant such an assurance.

Materials and methods

Samples

This study was carried out on 15 monovarietal EVOOs produced from autochthon varieties of olives (only cultivated in the Emilia-Romagna region and characterized by a genotype which differs greatly from those of other Italian cultivars) from orchard with a conventional agronomic system located in a circumscribed area of the Emilia-Romagna region (Brisighella, Ravenna), in the north-central of Italy. Specifically, 10 EVOOs obtained from olives belonging to the Nostrana of Brisighella cultivar (main and fundamental variety for obtaining the Brisighella PDO EVOO; codes NB1-NB10) and 5 produced from the Ghiacciolo cultivar (the secondary variety more frequently used; codes GH1-GH5) were analysed.

The cultivars Nostrana di Brisighella and Ghiacciolo were exclusively grown in Brisighella area; the first represents the main variety used for producing PDO 'Brisighella' olive oil (this *cv* that must

make up at least 90% of groves and minor varieties are admitted if not more than 10%). The Ghiacciolo cv besides being typical of the territory is very often used as olive tree pollinator (in the ratio of 1 of 5 of the total area); therefore, it was interesting to also include this variety in this study. These samples were collected within the production area of the "Brisighella" PDO from 14 olive orchards with different characteristics in terms of climatic and environmental conditions (e.g. altitude, geographical orientation and slope of the olive orchard). **Table 1** shows all relevant sample information. Olives were hand-picked at the optimal olive degree of ripening; a range of ripening index RI from 2.5 to 3.5 is recommended according to Rotondi et al. [16-17] for these varieties (from 25 October to 26 November); olives were immediately processed by a continuous industrial system (Alfa Laval NX X19 decanter, Alfa Laval s.p.a) equipped with a toothed discs crusher, a horizontal malaxator and three-phase phase decanter. The temperature (25°C) and time of malaxation (35 min) were standardized. EVOOs were filtered with a conventional filtration system using cellulose paper as filter aids in conjunction with filtration equipment (presses), bottled (250 ml dark glass bottles) and stored in thermostat at 10-12°C before chemical and sensory analyses.

Oil chemical quality indices

Basic quality parameters, including free acidity (FA), peroxide value (PV) and specific extinctions in UV (K_{232}, K_{270}) were assessed according to European Economic Community Regulations no. 2568/91 and its later modifications. Sensory analysis was performed by the Professional Committee of DISTAL (Department of Agricultural and Food Sciences of University of Bologna, recognized by the Italian Ministry of Agricultural, Food and Forestry Policies). Positive sensory descriptors (fruity, bitter and pungent) were evaluated according to the official procedure (EEC 2568/91 and following amendments). Moreover, evaluation of green notes and other positive attributes was carried out with reference to the list of descriptors established for PDO EVOOs, according to the IOC standards [11].

Fatty acid composition

The fatty acid composition was determined as fatty acid methyl esters (FAMEs) by gas chromatography (GC) analysis after alkaline treatment according to the official method [18].

HPLC analysis of phenolic compounds

The extraction of the phenolic fraction was performed according to the COI/T.20/Doc No 29 protocol, using an aqueous methanol solution and subsequent quantification by HPLC coupled to an UV detector at 280 nm. Syringic acid was used as the internal standard, while the content of the individually identified phenolic compounds was expressed as tyrosol (mg kg^{-1}). Chromatographic analysis was performed using a 1260 series HPLC instrument equipped with a quaternary pump (Agilent Technologies, Waldbronn, Germany) and a reverse phase C18 100A Kinetex column (2.6 μm, 100 x 3.00 mm I.D., Phenomenex, Torrance, CA, USA). The elution gradient was carried out with a solvent system of water/formic acid (99.5:0.5 v/v) as mobile phase A and acetonitrile as mobile phase B; the total run-time was 13 min and the gradient elution was as follows: from 0 to 3 min solvent B increased from 5% to 20%, at 4 min solvent B reached 40%, at 9 min solvent B reached 60%, and finally at 10 min solvent B at 100%; at 13 min, 5% solvent B was restored. The column was thermostated at 30°C and equilibrated for 5 min prior to each analysis; an injection volume of 5 μL and a flow rate of 0.7 mL min^{-1} were used. The main phenolic compounds were tentatively identified based on mass spectra using a mass spectrometer (MS, Agilent) in electrospray ionisation mode. The MS working conditions were: nebuliser gas pressure, 0.24 MPa; drying gas flow, 7 L min^{-1} at 300°C; capillary voltage, 2.5 kV. Nitrogen was used as a nebuliser and drying gas (Gaslab NG LCMS 20 generator, Equcien, Madrid, Spain). The MS was scanned within the m/z 100-900 range in the negative and positive ion mode.

Determination of volatile compounds

Analysis was performed by SPME/GC-MS according to the procedure described in Baccouri et al. [19]; SPME fibres were coated with a divinylbenzene/carboxen/polydimethylsiloxane

(DVB/CAR/PDMS) phase (50/30 μm, 2 cm long from Supelco Ltd., Bellefonte, PA, USA) and 4-methyl-2-pentanone (Fluka, Sigma-Aldrich, Steinheim, Germany) was added as an internal standard. Volatile compounds were identified and quantified by GC (Agilent 6890N) coupled with a quadrupolar MS (Agilent 5973N, Agilent Technologies). Analytes were separated on a column 30 m, 0.25 mm i.d., 1.00 μm f.t. (Phenomenex) coated with polyethylene glycol phase. The column temperature was held at 40°C for 10 min and increased to 200°C at 3°C min^{-1}. The ion source and transfer line were set to 180° and 230°C, respectively. Electron impact MS were recorded at 70 eV ionisation energy in the 20-250 a.m.u. mass range, 2 scans sec^{-1}. The identification of volatile compounds was obtained by comparison of their mass spectral data with the information from the NIST library (2005 version) and MS literature data. Volatile compounds were expressed as mg of internal standard per kg of oil.

Statistical analysis

XLSTAT 2011.1.03 version (Addinsoft, Paris, France) software was used to elaborate chemical data by analysis of variance (ANOVA) followed by Fishers, LSD post-hoc test ($p < 0.05$).

Results and discussion

Table 1 shows information related to irrigation, slope, altitude, orientation of orchards for each sample. Despite the Brisighella PDO product specification does not indicate mandatory characteristics (e.g. irrigation or not) or specific ranges concerning slope, altitude or orientation of orchards, this information was reported as a support of natural variability reinforcing the representativeness of sampling. Concerning chemical quality parameters, as reported in **Table 2**, all samples showed values under the respective limits established for EVOOs [18] and, specifically, under the stricter limits established by the product specification of Brisighella PDO [7]. These data confirmed the excellent quality of the raw material: healthy fruits, not attacked by *Bactrocera oleae*

or subject to prolonged storage before processing. All samples showed FA and PV values lower than the fixed limits for Brisighella PDO (0.5% and 13 mEq per kg, respectively). As suggested by official methods these basic quality parameters (FA and PV), together with spectrophotometric indices (K_{232}, K_{270}), are valuable olive oil freshness indices that confirm the good overall quality of these oils. The only exception was sample NB7 that presented a higher value (2.2) of K_{232} than the limit fixed for Brisighella PDO (2.0). The limit of K_{270} (1.6) reported for Brisighella PDO was erroneously indicated in the product specification; in fact, in accordance with the legislation on the quality characteristics of EVOO [18], this value must be not more than 0.22. Therefore, in the amendment, this typographical mistake has been corrected and the limit of 0.20 was inserted [20].

The fatty acid composition (**Table 2**) confirmed that all the samples belong to the EVOO category and highlights the high values of oleic acid (between 74.56% and 77.88%) and rather low (lower than 7.59%) values of the main polyunsaturated fatty acid, linoleic acid. Specifically, the oleic acid content ranged from 75.59 to 77.15% for samples produced from the Ghiacciolo cultivar and from 74.56 to 77.88% for samples from the Nostrana di Brisighella cultivar. Comparing these results with the historical analytical data of EVOOs certified as POD Brisighella in the period 2004-2012, commissioned by the certification control body (data not shown), it is possible to observe a progressive reduction in the percentage of oleic acid over time. A modification of the limit of the oleic acid content of Brisighella PDO EVOOs has been proposed in the product specification, reducing its minimum percentage from 75% to 73% [20]. This modification is a consequence of the change in the climate that has occurred in recent years as illustrated by the report of the Regional Agency for Prevention, Environment and Energy in the Emilia Romagna region (ARPA) (https://webbook.arpae.it/clima/index.html). Specifically, for the period 1961-2017, in Emilia-Romagna region, there was a significant trend in the increase in annual temperatures, both minimum and maximum, and more marked for maximum temperatures (0.25 °C/10 years and 0.45 °C/10 years, respectively). Very intense temperature anomalies were recorded especially during the summer; this occurrence affected the phases involved in oil accumulation (from fruit setting to fruit maturation).

The high temperature in the hot season promotes the desaturation of fatty acids and can result in decreased oleic acid content and a concomitant increase of linoleic acid [21-22]. The increase in temperature also elicits the anticipation of the harvest period because fruits reach the ripeness stage earlier; for this reason, the harvesting start date (5 of November), previously indicated in the product specification of Brisighella PDO [7], has been replaced with the indication to consider the status of the season and the state of ripening.

In this study, the entire set of samples (15 oils) was assessed with the aim to adapt the sensory description present in the product specification of Brisighella PDO [5] with the current regulation [11;23]. The intensities of principal positive attributes like olive fruity (green and/or ripe), bitter, pungent and secondary positive notes selected from the IOC list of descriptors for DO of EVOO [11], perceived both through olfactory and gustatory routes by the tasters, are reported in **Table 3**. The sensory profile of samples obtained from Ghiacciolo cultivar (GH1-GH5) showed a medium intensity of fruity, a medium-intense perception of bitter (values ranging from 5.5 to 6.4) and an intense sensation of pungent. Regarding the secondary positive notes, tomato was the most frequent descriptor indicated by the panel (3 samples out of 5: GH1, GH2 and GH5) followed by grass (2 samples of 5: GH3 and GH5) and almond (2 samples of 5: GH3 and GH4). The sensory profile of samples obtained from Nostrana di Brisighella cultivar (NB1-NB10) was very similar to the previous ones in terms of intensities of fruity, bitter and pungent, but different for the secondary positive attributes: these samples showed clear notes of artichoke (9 samples of 10: NB1-NB6 and NB8-NB10), grass (8 samples of 10: NB1-NB6, NB9 and NB10) and tomato (8 samples of 10: NB1-NB4, NB6-NB8 and NB10). These sensory profiles were also confirmed by Rotondi et al. [24] and by the data collected for many Italian monocultivar oils (http://www.olimonovarietali.it). In Emilia-Romagna region there are other widely grown cultivars (Correggiolo, Moraiolo and Leccino) also cultivated in other Italian regions having a genotype similar than the same cultivars found in the other regions. The varieties Nostrana di Brisighella and Ghiacciolo, instead, are native of Emilia-Romagna region and confer unique characteristics upon the product; the olive growers of the Brisighella area

have found in these cultivars the characteristics that enable them to provide the consumer with an exclusive product thanks to the strong link between the production area and the locally predominant olive cultivars. These varieties over the centuries has adapted their specific phenological characteristics to survive in an olive growing area located at the limits of where olives can be grown. VOOs can be considered different from other vegetable oils since, by refining, these latter lose most of the minor components such as volatile molecules and phenolic compounds that are responsible for sensory perceptions.

Specific classes and single volatile compounds determine the perception of olfactory notes (positive and negative); combination of volatile compounds, present in different amounts in VOOs, represents a sort of digital fingerprint and, therefore, this analysis could allow evaluation of their quality and typicality.

Table 4 shows the phenolic content and volatile compounds determined by HPLC-DAD-MS and by SPME/GC-MS, respectively. The total content in phenolic compounds was determined as the sum of all the individual molecules identified and quantified (hydroxytyrosol; tyrosol; a tyrosol derivative; caffeic acid, hydroxycinnamic acid derivatives; decarboxymethyl oleuropein aglycone; decarboxymethyl ligstroside aglycone; oleuropein aglycone; ligstroside aglycone; pinoresinol and acetoxypinoresinol) resulting in a minimum of 256.34 (NB4) up to a maximum of 433.83 (NB5) mg of tyrosol kg^{-1} of oil.

Considering the recent possibility of indicating on the label the health claim "olive oil polyphenols contribute to the protection of blood lipids from oxidative stress", the total content of phenolic derivatives of hydroxytyrosol and tyrosol (free and bond forms) present in 20 g of oil was calculated [25], considering only the amount of the following molecules: hydroxytyrosol, tyrosol, a tyrosol derivative, decarboxymethyl oleuropein aglycone, decarboxymethyl ligstroside aglycone, oleuropein aglycone and ligstroside aglycone). Most of the samples included in the present study, excluding four (GH3, NB3, NB4, NB7), reached the level required by the EU for the health claim. In a recent study

by Antonini et al. [26] on Italian PDO EVOOs, Brisighella PDO oil (*n* =4) showed the highest phenolic content and met the EU health claim for phenol concentration.

The volatile compounds identified and quantified in the headspace of EVOOs and responsible for their flavour are reported in **Table 4**. In general, the aromatic profile of samples was very similar, however, samples produced from Nostrana di Brisighella showed values of total C_6-LOX content higher than those obtained from Ghiacciolo oils with mean values of 4.7 and 3.2 mg expressed as 4-methyl-2-pentanone per kg of oil, respectively. Among C_6-LOX compounds, those most quantitatively present were aldehydes generally associated with positive sensory notes like ‘‘green’’, ‘‘almond’’ and ‘‘cut grass’’ [27-28]. Only for three samples of Nostrana di Brisighella (NB6, NB8, NB10) the most representative C_6 compounds derived from LOX pathway were alcohols. In addition, all samples contained reasonable amounts of C_5 volatile compounds (mainly represented by ketones), which also can contribute to the positive attributes of olive oil, providing pungent sensations and correlating with bitterness [27]. A comparison with literature data on the volatile and phenolic composition of Tunisian monovarietal EVOOs [29-30], in which analytes were quantified by the same analytical protocols applied in this study, showed that C_6 aldehydes and alcohols were the most abundant compounds contributing favourably to the aroma of samples. However, Tunisian samples exhibited lower amounts of C_5 ketones and the absence of pentene dimers, both affecting EVOO aroma [31]. It is also necessary to keep in mind that the sensory perception of a volatile compound depends on its odour activity value (OAV, ratio of concentration to odour threshold) and on enhancement or suppression effects of different odourants and other non-volatile components present in oils. Due to their high odour threshold, pentene dimers seem to have less sensory significance than the other C_5 compounds such as ketones; these latter positively correlated with phenolic compounds and negatively with hexanal and have been proposed by some authors as quality-freshness markers for VOOs [31-32]. Regarding the phenolic profile, the differences were mainly in terms of identified compounds: hydroxytyrosol was not detected and tyrosol was present only in low quantities. As a quantitative perspective, the total phenol content of Brisighella PDO EVOOs was similar than those

reported by Nsir et al. [30] only for oils produced by Sayali olives at the green stages of maturation that, in some cases, fulfilled the conditions of the health claim.

Conclusions

Typical foods as PDO products are closely linked with the physical environment, culture and tradition of the place of production; they result from an evolution process of work practices and knowledge that need to be handed down and innovated over time. This work, through the update of the Brisighella PDO product specification, contributes to the product innovation and helps the Consortium in promoting, developing and informing consumers by providing a tool to distinguish Brisighella PDO EVOO in the market.

The proposed modifications approved by EU in 2016 [20] aimed to: i) update selected parameters taking into account the significant increase in temperature in Emilia-Romagna region over recent years (the decrease of the minimum oleic acid content from 75% to 73% as a consequence of the anticipation of the date of harvest); ii) underline the high product quality (e.g. the reduction of the free acidity limit from 0.5 % to 0.3 %); iii) adapt the product specification to the current legislation in the sector (specifically for sensory analysis adopting the median values for fruity, bitter, pungent and for secondary and typical notes in accordance with the IOC method provided for DO products), and finally; iv) promote the health benefits of Brisighella PDO consumption based on its content in biophenols (the possibility to indicate the related health claim on the label).

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Sample code	Variety	Irrigation	Slope (%)	Altitude (hasl)	Orientation
GH1	Ghiacciolo	yes	15	140	W
GH2	Ghiacciolo	no	40	300	SE
GH3	Ghiacciolo	no	15	175	S
GH4	Ghiacciolo	no	30	310	SE
GH5	Ghiacciolo	no	25	220	E
NB1	N. di Brisighella	no	25	180	SW
NB2	N. di Brisighella	no	40	300	SE
NB3	N. di Brisighella	yes	18	120	NE
NB4	N. di Brisighella	no	10	370	NE
NB5	N. di Brisighella	no	33	310	E
NB6	N. di Brisighella	yes	50	130	S
NB7	N. di Brisighella	no	35	225	E
NB8	N. di Brisighella	no	47	140	NW
NB9	N. di Brisighella	no	12	370	NW
NB10	N. di Brisighella	yes	3	140	NW

Table 1. Information, features and coding of extra virgin olive oil (EVOOs) samples.

	FA	PV	K_{232}	K_{270}	C18:1	C18:2
GH1	0.2	10^{e-i}	1.6^{cd}	0.13^{de}	77.15^{bc}	6.09^{f}
GH2	0.2	13^{a}	2.0^{a}	0.14^{b-d}	75.59^{ef}	7.53^{a}
GH3	0.2	12^{a-c}	1.4^{e}	0.16^{a}	76.74^{bd}	6.55^{d}
GH4	0.2	9^{i}	1.7^{cd}	0.15^{a-c}	76.40^{d}	6.69^{c}
GH5	0.2	10^{f-i}	1.4^{e}	0.15^{ab}	76.53^{cd}	7.59^{a}
NB1	0.2	11^{b-d}	1.7^{cd}	0.11^{g}	74.56^{g}	6.90^{b}
NB2	0.2	11^{b-e}	1.2^{f}	0.12^{d-f}	76.38^{d}	5.89^{g}
NB3	0.2	12^{ab}	1.6^{d}	0.12^{e-g}	75.68^{ef}	6.27^{e}
NB4	0.2	9^{g-i}	1.6^{cd}	0.11^{fg}	76.46^{d}	5.92^{g}
NB5	0.2	10^{d-g}	1.9^{b}	0.15^{a}	76.46^{d}	5.92^{g}
NB6	0.2	11^{b-e}	1.4^{e}	0.13^{de}	75.67^{ef}	5.76^{h}
NB7	0.2	10^{d-h}	2.2^{a}	0.13^{de}	75.42^{f}	5.86^{gh}
NB8	0.2	9^{hi}	1.7c	0.12^{d-f}	76.11^{de}	5.44^{i}
NB9	0.2	10^{f-i}	1.2^{f}	0.13^{cd}	77.34^{ab}	5.21^{j}
NB10	0.2	11^{c-f}	2.0^{ab}	0.13^{b-d}	77.88^{a}	4.78^{k}
(UE) 2095/2016	≤ 0.8	≤ 20	≤ 2.5	≤ 0.22	55-83%	2.5-21%
(EC) 1263/1996	≤ 0.5	≤ 13	≤ 2.0	≤ 1.60*	≥ 75%	≤ 8.00%

*value erroneously indicated in the product specification.

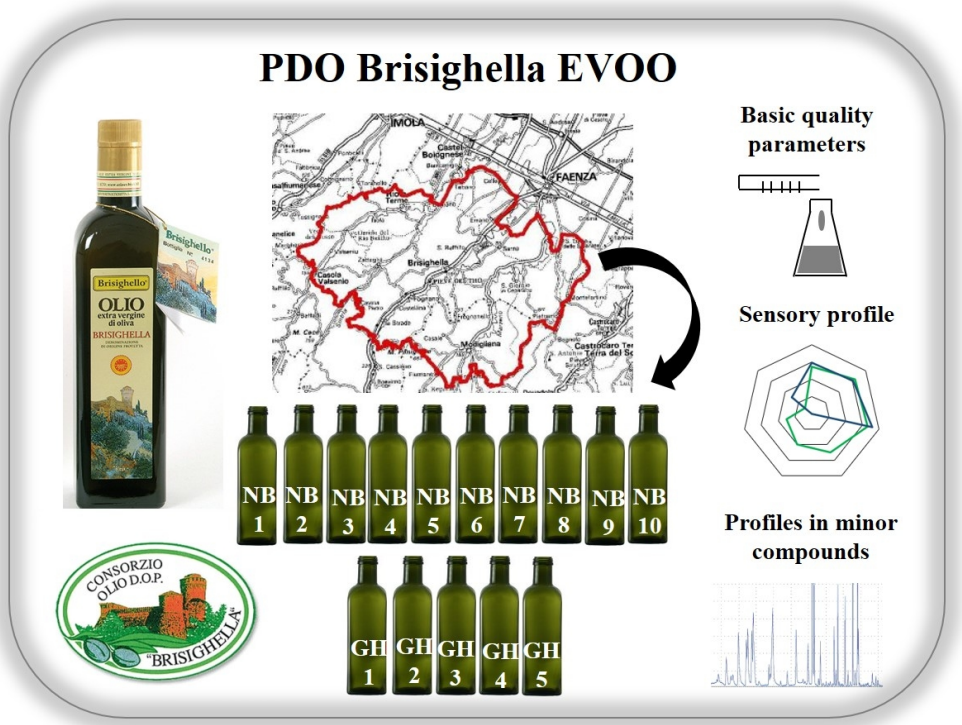
Table 2. Free acidity, FA (expressed as g oleic acid per 100 g of oil); peroxide value, PV (expressed as meq of active oxygen per kg of oil); K_{232}, K_{270} (expressed as specific extinctions); oleic acids (C18:1) and linoleic acid (C18:2) expressed as a percentage of each fatty acid of the total. All results are reported as the mean of three replicates. Different letters in the same column indicate significant differences (Fisher LSD, $p < 0.05$).

	Fruity	Bitter	Pungent	Artichoke	Grass	Tomato	Almond
GH1	5.5	5.9	6.3	4.3	n.d	4	n.d
GH2	5.9	6.1	7.2	n.d	n.d	4.7	n.d
GH3	6.0	6.4	7.0	n.d	4.3	n.d	3.5
GH4	5.2	5.5	6	n.d	n.d	n.d	4
GH5	5.1	6.4	6.6	n.d	4.6	4.3	n.d
NB1	5.4	6.4	6.6	5	4	3	n.d
NB2	4.8	5.5	6.1	4.5	4.5	3.5	n.d
NB3	4.8	5.7	6	5.4	4	3.5	n.d
NB4	5	5.7	5.6	5	4.5	3	n.d
NB5	5.6	6.7	6.1	3	3	n.d	3.2
NB6	5.1	6.2	6.7	4.8	4	3.7	n.d
NB7	4.5	5.5	5.9	n.d	n.d	4.5	1
NB8	5.5	6.3	6.5	4.5	n.d	4	n.d
NB9	6.1	6.7	6.6	4.5	5	n.d	n.d
NB10	4.5	6.6	5.8	3.0	3.0	3.0	n.d

Table 3. Sensory results (median values) estimated by the Professional Committee of DISTAL (Department of Agricultural and Food Sciences of University of Bologna, recognized by the Italian Ministry of Agricultural, Food and Forestry Policies).

	Total Phenols	Health Claim	Aldehydes C_6 Lox	Alcohols C_6 Lox	Total C_6 Lox	Ketones C_5 Lox	Alcohols C_5 Lox	Pentenic dimers	Total C_5 Lox
GH1	385.13^{cd}	6.0^{b-d}	1.36^{g}	0.81^{f}	2.17^{j}	0.48^{e-g}	0.35^{de}	0.58^{e}	1.42^{de}
GH2	346.48^{e-g}	5.4^{e-g}	2.32^{ef}	1.67^{d}	3.99^{f}	0.81^{c}	0.61^{c}	1.37^{b}	2.80^{b}
GH3	316.90^{gh}	4.9^{g-i}	2.66^{de}	0.44^{g}	3.10^{gh}	0.56^{d-f}	0.23^{g}	0.38^{gh}	1.16^{e}
GH4	395.51^{b-d}	6.0^{bc}	2.91^{d}	1.25^{e}	4.16^{ef}	0.86^{c}	0.27^{fg}	0.96^{d}	2.10^{c}
GH5	417.51^{ab}	6.2^{ab}	1.39^{g}	1.06^{e}	2.45^{ij}	0.46^{fg}	0.26^{fg}	0.43^{f-h}	1.15^{e}
NB1	396.24^{b-d}	6.4^{ab}	3.58^{c}	2.61^{b}	6.20^{b}	1.20^{a}	0.73^{b}	1.13^{c}	3.06^{ab}
NB2	351.39^{ef}	5.5^{d-f}	2.51^{de}	1.11^{e}	3.62^{fg}	0.58^{de}	0.37^{df}	0.53^{ef}	1.48^{d}
NB3	293.22^{h}	4.5^{ij}	3.86^{bc}	0.82^{f}	4.68^{de}	0.42^{g}	0.25^{fg}	0.58^{e}	1.25^{de}
NB4	256.34^{i}	4.0^{j}	8.08^{a}	2.55^{b}	10.63^{a}	0.87^{c}	0.57^{c}	1.69^{a}	3.14^{a}
NB5	433.83^{a}	6.7^{a}	2.45^{e}	0.82^{f}	3.27^{gh}	0.57^{d-f}	0.38^{d}	0.321^{h}	1.27^{de}
NB6	378.11^{de}	6.0^{bc}	1.25^{g}	1.52^{d}	2.78^{hi}	0.61^{d}	0.41^{d}	0.43^{f-h}	1.45^{d}
NB7	304.06^{h}	4.6^{hi}	4.02^{b}	0.83^{f}	4.85^{d}	0.51^{d-g}	0.26^{fg}	0.50^{e-g}	1.27^{de}
NB8	374.30^{de}	5.6^{c-e}	2.04^{f}	3.46^{a}	5.50^{c}	1.02^{b}	0.85^{a}	1.46^{b}	3.33^{a}
NB9	412.77^{a-c}	6.4^{ab}	1.38^{g}	0.80^{f}	2.18^{j}	0.57^{d-f}	0.31^{ef}	0.38^{gh}	1.26^{de}
NB10	336.21^{fg}	5.1^{f-h}	1.26^{g}	1.96^{c}	3.22^{gh}	0.49^{e-g}	0.40^{d}	0.62^{e}	1.51^{d}

Table 4. Phenolic compounds determined by HPLC-DAD/MSD and expressed as mg of tyrosol per kg of oil. Total phenols: sum of all the individual molecules identified and quantified (hydroxytyrosol; tyrosol; a tyrosol derivative; caffeic acid, hydroxycinnamic acid derivatives; decarboxymethyl oleuropein aglycone; decarboxymethyl ligstroside aglycone; oleuropein aglycone; ligstroside aglycone; pinoresinol and acetoxypinoresinol). Health claim: mg of bonds and free forms of hydroxytyrosol and tyrosol (hydroxytyrosol, tyrosol, a tyrosol derivative, decarboxymethyl oleuropein aglycone, decarboxymethyl ligstroside aglycone, oleuropein aglycone and ligstroside aglycone) present in 20 g of oil. Volatile compounds associated with flavour (aldehydes C_6, alcohols C_6, ketones C_5, alcohols C_5, penten dimers), expressed as mg of 4-methyl-2-pentanone per kg of oil. All results are reported as the mean of three replicates. Different letters in the same column indicate significant differences (Fisher LSD, $p < 0.05$).



204x156mm (150 x 150 DPI)

Recent amendment to product specification of Brisighella PDO (Emilia Romagna, Italy)