Food Chemistry 139 (2013) 949–954

Contents lists available at SciVerse ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Extra virgin olive oil bitterness evaluation by sensory and chemical analyses

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ARTICLE INFO

Article history:
Received 27 September 2012
Received in revised form 18 January 2013
Accepted 22 January 2013
Available online 11 February 2013

Keywords:
Bitterness
Extra virgin olive oil
Predictive model
Sensory evaluation
Total phenol content

ABSTRACT

An experimental investigation was performed on blend extra virgin olive oils (EVOOs) from different cultivars and EVOO from different olive monovarieties (*Coratina*, *Leccino, Maiatica*, *Ogliarola*) with the aim to evaluate the possibility of estimating the perceived bitterness intensity by using chemical indices, such as the total phenol content and the compounds responsible for oil bitterness measured spectrophotometrically at 225 nm (K_{225} value), as bitterness predictors in different EVOO. Therefore, a bitterness predictive model, based on the relationship between the perceived bitterness intensity of the selected stimuli and the chosen chemicals parameters has been built and validated. The results indicated that the oil bitterness intensity could be satisfactorily predicted by using the K_{225} values of oil samples.

1. Introduction

Bitterness, pungency and astringency are sensory attributes of extra virgin olive oils (EVOOs), often positively linked to the presence of phenolic compounds in the medium (Mateos, Cert, Pérez-Camino, & García, 2004). Bitterness is generally considered as a positive sensorial attribute of the oil and enhances the overall flavour with notes related to unripe olive fruit (Inarejos-Garcia et al., 2009). The secoridoid derivatives are the main compounds responsible for the bitter taste of EVOO, such as oleuropein and ligstroside derivates (De Stefano, Piacquadio, Servili, Di Giovacchino, & Sciancalepore, 1999; Esti, Contini, Moneta, & Sinesio, 2009; Inajeros Garcia et al., 2009; Mateos et al., 2004). In particular, it has been demonstrated that oleuropein is the most abundant compound in virgin olive oils and that its concentration could be related to different types of cultivars (Perri, Raffaelli, & Sindona, 1999). Moreover, varieties with smaller drupes have shown a higher level of oleuropein (Amiot, Fleuriet, & Macheix, 1986; Škevin et al., 2003).

Depending on the type of phenols present, rather than on the total phenol content, the intensity of bitterness of olive oils can be extremely variable (high or low). Therefore, it is important to establish the optimal level of bitterness in EVOO, depending on several factors, such as harvesting time, oil extraction system and olive variety (Gawel & Rogers, 2009; Koseoglu & Unal, 2008; Škevin et al., 2003).

Also, due to the positive contribution of the phenolic compounds to the olive oil oxidative stability and human health, consumers are increasing their consumption of oils with high bitterness intensity (Inarejos-Garcia et al., 2009). As a result, bitterness evaluation is becoming an important area in olive oil research.

The standard method for analysing the bitter taste of EVOO is sensory analysis using a trained panel (EC Reg. 796/02). However, sensory evaluation is not simple, being a rather time-consuming process, because a permanent staff of trained tasters is required. For this reason, methods of bitterness evaluation based on physicochemical determinations could be more useful for the industry (Beltràn, Ruano, Jimenez, Uceda, & Aguilera, 2007; Mateos et al., 2004). An objective method that would permit measurements of bitterness intensity is certainly preferable, especially if the results of both sensory and analytical data are in statistical agreement (Inarejos-Garcia et al., 2009). Furthermore, a predictive model of olive oil bitterness intensity may have practical applications in the oil industry.

In order to evaluate the bitter taste in EVOO, Gutiérrez Rosales, Perdiguero, Gutiérrez, and Olias (1992) have proposed a simple analytical method based on the extraction of the bitter compounds measured by spectrophotometric determination at 225 nm, reporting a good correlation with the bitter taste evaluated by sensory analysis. Moreover, Beltràn et al. (2007) have proposed an easy method to estimate the oil bitterness intensity without any sensory evaluation by measuring the total phenol content. However, in both cases, either by using the total phenols (Beltràn et al., 2007) or the bitter compounds measured at 225 nm (Gutiérrez Rosales et al., 1992), a predictive model has not been validated with unknown oil samples. In addition, both Gutiérrez Rosales et al. (1992) and Beltràn et al. (2007) used a five-point structured scale for oil sensory evaluation; the use of this procedure only allows discrimination between oils markedly different in terms of bitterness intensity.

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http://dx.doi.org/10.1016/j.foodchem.2013.01.098

Taking into account these assumptions, the aim of this work was to evaluate the possibility of estimating the perceived bitterness intensity by using chemical indices, such as total polyphenol content, and the compounds responsible for oil bitterness measured spectrophotometrically at 225 nm, as bitterness predictors of different EVOO. Furthermore, in order to build up and validate a bitterness predictive model, the relationship between the perceived bitterness and the chosen chemical parameters has been systematically investigated. In this study, the use of K_{225} value as bitterness predictor has been reassessed, taking into account the relative simplicity of this analytical procedure, compared with other methods recently proposed, such as fluorimetry, HPLC coupled with fluorescence and diode array detection systems (Inarejos-Garcia et al., 2009). The adoption of a simple prediction method is useful in order to enable a fast and continuous control of the intensity of the bitterness, which is considered a key attribute of oil acceptability. Moreover, in order to improve the predictive capacity of the model, sensory analysis has been performed using a non-structured linear scale, according to the official method proposed for evaluating olive oil quality, which allows a better differentiation between oil samples and increases the discriminant ability of panellists.

2. Materials and methods

2.1. Experimental design

The experimental design consisted of four stages:

1. A first stage to study the perceived bitterness intensity of selected EVOO samples (training set sample selection) according to the official procedure (EC Reg. 796/2002);
2. A second stage to determine the chemical indices of oil samples in terms of total phenol content and K_{225} value;
3. A third stage to build up a bitterness predictive model based on the relationship between the perceived bitterness intensity of the selected sensorial stimuli and chemical parameters (building the predictive model);
4. A fourth stage to validate the predictive capacity of the model by comparing predicted and measured bitterness intensities in a test set consisting of unknown oil samples (predictive model validation).

2.2. Sampling

A set named "sample set" consisting of 35 commercial EVOO samples, produced in the year 2010 in the Basilicata region (Italy), was prepared. In order to characterise the oils, to study the perceived bitterness and also to build up the predictive model, chemical and sensory analyses were performed. Furthermore, a set called "test set" consisting of 10 oil samples (2 for each group) was prepared in order to validate the predictive model. Therefore, a total of 45 oil samples was evaluated.

The oil samples were obtained from olive fruits (*Olea europea* L.) of different monovarieties (9 for each cultivar): *Coratina*, *Leccino*, *Maiatica*, *Ogliarola* and 9 blend oil samples obtained from different cultivars.

The olives were processed by a two-phase centrifugal extraction. All samples were stored at 15 °C in darkness using topaz bottles sealed under N_2 prior to analysis.

2.3. Chemical analyses

The total polyphenol content in EVOO was determined following the method proposed by Favati, Caporale, and Bertuccioli (1994). The compounds responsible for oil bitterness were evaluated spectrophotometrically at 225 nm with a Cary 1E UV–Visible Spectrophotometer (Varian, Leini, Italy) according to the method proposed by Gutiérrez Rosales et al. (1992).

All solvents and reagents were of analytical grade and were purchased from Carlo Erba (Milano, Italy). Solid-phase extraction (SPE) C18 cartridges (6 mL) were obtained from Supelco (Milano, Italy). All the analyses were run in duplicate.

2.4. Sensory analysis

2.4.1. Training session

A total of 16 recruited subjects participated in the experiments. The subjects were trained to recognise and rate the perceived bitterness intensity using quinine monohydrochloride dehydrate (Sigma Aldrich, Milano, Italy) standard solutions at concentrations of 0.025, 0.037 and 0.050 g/L. During the training session the subjects were asked to rate the perceived bitterness by using a linear non-structured scale from 0 to 10 (10 cm), according to the official procedure (EC Reg. 796/2002). Eight subjects, 3 males and 5 females, (ages 20–30) took part in the experiments. Subjects were selected from the recruited panel of 16 subjects on the basis of their performance in the training session. Selected subjects were also trained in the recognition of bitter attributes of virgin olive oil by the evaluation of standard samples (COI, 1996) and agreed on the definition of this attribute, described as the typical taste of an oil produced from unripe olives.

2.4.2. Evaluation of the "sample set"

Eight selected subjects were asked to rate the perceived bitterness of 35 oil samples. Each sample was evaluated in duplicate. Subjects participated in a total of 10 sessions; in each session 7 samples were presented. There was a 10-min interval between two sub-sessions consisting of 4 and 3 samples respectively. Within each session the presentation order of samples was balanced for first order and carry-over effects and 15 mL of each sample were tasted according to the official procedure (EC Reg. 796/2002). Oils were served in coloured tasting glasses to mask colour differences, thus eliminating the visual factor. The temperature of the oils was kept constant (28 ± 2 °C). Assessors were instructed to take the oil sample in the mouth and to allow the oil to reach the back of the tongue. Subjects were then asked to rate the perceived bitterness using a non-structured linear scale (10 cm). Between the evaluation of two samples, subjects were asked to rinse their mouths with distilled odourless water for 45 s, to have some plain crackers for 30 s and finally to rinse their mouths with water for a further 45 s. The evaluations were performed in individual booths under red light.

Two weeks after the last session of the "training set" sample evaluation, subjects participated in a further experiment in which the bitterness of the "test set" samples was evaluated. The experiments consisted of two repetitions; in each session five samples were presented. The subject took part in a total of four sessions. Samples were evaluated under the same conditions as previously described.

2.5. Statistical analysis

Sensory data were collected and elaborated by using the software FIZZ (ver. 1.31, Biosystèmes, Couternon, France). Data were processed by mixed model analysis of variance (ANOVA) assuming subjects as random effect; moreover, the least significant difference (LSD) test was performed to compare the means ($p \leqslant 0.05$). Regression analysis was also applied to the data. All statistical procedures were computed using the statistical package SYSTAT for Windows (ver. 10, 2003) (Systat Software, Chicago, IL).

Table 1
Perceived bitterness intensity of oil samples for each cultivar (mean value ± S.D.).

Coratina	Ogliarola	Maiatica	Leccino	Blend
1.60 ± 0.30[a]	1.24 ± 0.93[a]	0.76 ± 0.24[a]	0.76 ± 0.35[a]	0.70 ± 0.33[a]
4.00 ± 0.70[b]	1.44 ± 1.45[a]	1.00 ± 0.43[ab]	1.24 ± 0.11[ab]	1.57 ± 0.58[ab]
4.00 ± 0.55[b]	1.59 ± 0.20[a]	1.13 ± 0.21[abc]	1.60 ± 0.73[bc]	1.58 ± 0.33[ab]
4.88 ± 0.33[c]	1.90 ± 0.46[a]	1.29 ± 0.88[bc]	1.90 ± 0.92[bc]	2.13 ± 1.14[bc]
6.51 ± 0.50[c]	2.89 ± 0.25[b]	1.30 ± 0.29[bc]	2.13 ± 0.93[c]	2.93 ± 1.58[cd]
7.03 ± 1.04[d]	4.41 ± 0.76[c]	1.33 ± 0.45[bc]	3.50 ± 0.46[d]	3.30 ± 0.36[d]
7.50 ± 0.86[d]	5.50 ± 0.61[d]	1.51 ± 0.29[c]	4.50 ± 0.41[e]	4.38 ± 0.84[e]

Data followed by different letters in the same column are significantly different (LSD test at $p \leqslant 0.05$).

3. Results and discussion

3.1. Sensory and chemical evaluation of extra virgin olive oil samples

Initially, in order to determine the perceived bitterness intensity, a total of 35 EVOO was tested by trained subjects and by using a linear non-structured scale (10 cm), according to the official procedure (EC Reg. 796/2002). The use of the official method produces data for the characterisation of oil samples and the monitoring of their compliance with regulatory requirements and production specifications. Furthermore, the use of a non-structured linear scale allows a better differentiation among oil samples and increases the discriminant ability of panellists. Some authors (Beltràn et al., 2007; Gutiérrez Rosales et al., 1992; Mateos et al., 2004) used a five-point category scale in the building up of bitterness predictive models; however, this scale is not suitable for an adequate discrimination of olive oils characterised by high bitterness levels, because when using a five-point scale the panellist has a lower and less precise possibility to assess the intensity of the evaluated parameter.

Data were treated separately by cultivar. The results from the mixed ANOVA model performed on the bitterness intensity ratings of the oil samples showed a significant effect of the samples on the bitterness intensity ratings ($p \leqslant 0.001$), while the replicate did not show a significant effect ($p \geqslant 0.05$). Mean bitterness intensity ratings and their standard deviation are reported in Table 1. For each cultivar sample set, the mean intensity ratings of the seven samples were significantly different ($p \leqslant 0.05$) and could be divided into four groups. Mean intensity ratings ranged from 0.7 (blend sample) to 7.5 (*Coratina* sample), indicating that the perceived strength of bitterness ranges, on average, from very weak to very strong. The mean intensity ratings, within each cultivar sample, with the exception of the Maiatica oils, covered a wide range. Thus, the number of test set samples was assumed to be sufficient to build up the bitterness predictive model.

The oil samples were also chemically characterised in terms of total phenol content and compounds responsible for oil bitterness evaluated spectrophotometrically at 225 nm (K_{225} value). The results reported in Table 2 show a great variability in the measured parameters. The oils obtained from *Coratina* variety were characterised by a high content of total polyphenols, more than 400 ppm for most of the samples analysed, according to the literature for this cultivar (Caponio, Gomes, & Pasqualone, 2001; Clodoveo, Delcuratolo, Gomes, & Colelli, 2007; Rotondi, Alfei, Magli, & Pannelli, 2010), while those obtained from *Leccino* cv had an average total phenol content lower than 400 ppm, as also reported in the literature (Rotondi et al., 2010). The highest phenolic content was detected in the Coratina oil samples (mean 456 mg/L), while the blend samples (mean 198 mg/L) had the lowest content. Regarding the bitterness intensity, the highest score was found also in *Coratina* oil samples (mean 5.7), while the lowest was in *Maiatica* oils (mean 1.4). These data confirm that while bitterness is affected by the phenols present in the oil, its intensity cannot be assessed by a simple measurement of the total phenol content. In fact, with the exception of EVOO from *Coratina* cultivar (Clodoveo et al., 2007; Rotondi et al., 2010), the highest content of total phenols is not even correlated with the highest perception of bitterness (Esti et al., 2009).

As reported by others (Bendini, Cerretani, Salvador, Fregapane, & Lercker, 2009; Koseoglu & Unal, 2008; Škevin, 2003), different factors, such as olive variety, climatic conditions, fruit ripeness, storage conditions of olive fruit, technological processing of oil, the olive cultivar, as well as the harvesting time, had a statistically significant influence on the level of total phenols, *o*-diphenols and also on the intensity of bitterness, with the ripeness of olive fruits exerting a greater effect than the cultivar itself. Gambacorta et al. (2010) also highlighted the influence of maturation index, storage time, stoning and malaxation time on phenolic content, reporting that the highest concentration usually corresponds to the lowest maturation index values. As far as the phenolic profile is concerned, the oils from olives picked at low maturation index showed higher contents of all the compounds than the corresponding oils deriving from high maturation index olives.

Cultivar effect on the chemical parameters and the perceived bitterness was also investigated. The results of one-way ANOVA model have shown a significant effect of cultivar on K_{225} value and bitterness ratings (Table 2). The significant effect of the cultivar on bitterness intensity has been also confirmed by Rotondi et al. (2010), who showed a prevalent effect of the cultivar on the sensory profile of monovarietal oils. A significant effect of olive variety on the bitterness intensity evaluated spectrophotometrically has been also reported by other authors (Ilyasoglu, Ozcelik, Van Hoed, & Verhe, 2010; Škevin et al., 2003). Conversely, the cultivar did not significantly affect the total phenol content of oils (Table 2), even if a significant effect of olive variety on the level of total polyphenols has been reported (Ilyasoglu et al., 2010; Rotondi et al., 2010; Škevin et al., 2003).

Table 2
Effect of cultivar on the total phenol content, bitterness intensity and K_{225} value of EVOO samples ($n = 35$).

Cultivar	Total phenol content[1] (mg/L)		Bitterness intensity[2]		K_{225} value[3]	
	Mean	Range	Mean	Range	Mean	Range
Coratina ($n = 7$)	456 ± 53[*,a]	249–645	5.07 ± 0.8[a]	1.60–7.50	0.40 ± 0.05[a]	0.17–0.60
Ogliarola ($n = 7$)	382 ± 27[a]	237–448	2.71 ± 0.6[b]	1.24–5.50	0.24 ± 0.04[b]	0.15–0.45
Maiatica ($n = 7$)	330 ± 19[a]	255–378	1.19 ± 0.09[c]	0.76–1.50	0.12 ± 0.01[c]	0.06–0.16
Leccino ($n = 7$)	352 ± 41[a]	219–519	2.24 ± 0.5[bc]	0.76–4.50	0.21 ± 0.03[bc]	0.07–0.32
Blend ($n = 7$)	299 ± 37[a]	190–427	2.36 ± 0.5[bc]	0.70–4.38	0.21 ± 0.03[bc]	0.06–0.35

Data followed by different letters in the same column are significantly different (LSD test at $p \leqslant 0.05$).
[1] $F = 1.559$, $p = 0.206$.
[2] $F = 5.083$, $p = 0.002$.
[3] $F = 4.338$, $p = 0.006$.
[*] Standard error.

Taking into account the significant effect of cultivar on bitterness intensity and K_{225} value, it is reasonable to assume that cultivar probably influences the composition of the phenolic fraction, which is strongly related to the bitterness intensity. Therefore, this result could confirm the suitability to use as bitter predictor an index which considers the oil phenolic composition, rather than the total phenol content. Moreover, it is evident that total phenol content and K_{225} value could provide different information for better EVOO classification.

3.2. Building predictive model

In this study, building of the predictive model was performed by relating K_{225} values to bitterness intensity mean scores. Furthermore, considering that this chemical parameter was previously used as a perceived bitter intensity predictor (Beltràn et al., 2007), the predictive model also related the total phenol content to bitterness intensity mean scores.

In our preliminary test, relating K_{225} values and bitterness scores, similar correlations using either linear or polynomial models were found. However, taking into account the results of Inarejos-Garcia et al. (2009) and considering that correlation between bitterness and bitter compounds in various products is commonly studied by simple linear models in the case of one independent variable (Robichaud & Noble, 1990; Rousseff, 1990), a simple linear model has been chosen, obtaining a good correlation (r^2 = 0.97). In fact, predictive models have been successfully built using simple linear models for other foods, such as coffee and beer (Robichaud & Noble, 1990; Rousseff, 1990).

3.2.1. Predictive model based on total phenol content

The relationship between total phenol content and perceived bitterness intensity was considered; in particular, the regression was significant but associated with a low r^2 (0.42) and high standard error (1.27) (Fig. 1). The goodness of fit was also estimated. Residual values reached 2.33 (as absolute value) for regression obtained using phenolic concentration as bitterness predictor. This value represents a high prediction error, equal to 23.3%, considering the used scale (10 cm). Conversely, several authors (Beltràn et al., 2007; Busch, Hrnicirik, Bulukin, Boucon, & Mascini, 2006; Caporale, Policastro, & Monteleone, 2004; Koseoglu & Unal, 2008; Mateos et al., 2004; Rotondi et al., 2010; Suárez, Romero, Ramo, & Motilva, 2011) have found a strong direct relationship between total phenolic content and perceived bitterness intensity.

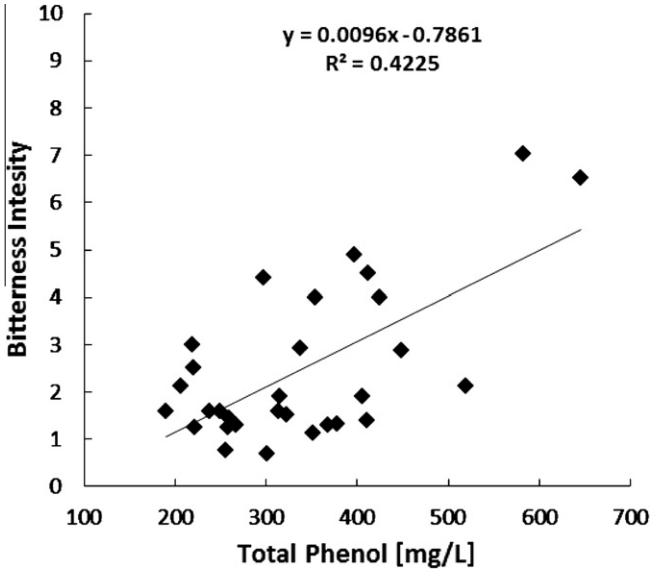

Fig. 1. Relationship between total phenol content (mg/L) and perceived bitterness intensity.

Although it is clearly known that polyphenols are the main contributors to olive oil bitterness and astringency, the contribution of each individual polyphenol to the total bitterness is not yet clear; a strong correlation between the concentration of secoridoid derivates of hydroxytyrosol and bitterness has been found, but only if one olive variety is used or in oils in which these compounds are the main components (Andrewes, Busch, De Joode, Groenewegen, & Alexandre, 2003).

The bitterness of EVOO is chemically associated with the sum of the contents of two secoridoid derivatives of hydroxytyrosol: the dialdehydic form of elenolic acid linked to hydroxytyrosol, (3,4-dihydroxyphenylethanol-elenoic acid, 3,4-DHPEA-EDA), and an isomer of oleuropein aglycon (3,4-dihydroxyphenylethanol, 3,4-DHPEA-EA). In fact, a good correlation between oil bitterness and content of hydroxytyrosol secoridoid derivatives has been found; in particular a strong correlation between the 3,4-DHPEA-EDA and 3,4-DHPEA-EA content and bitterness intensity (Garcia, Yousfi, Mateos, Olmo, & Cert, 2001; Gutiérrez Rosales, Ríos, & Gómez-Rey, 2003; Mateos et al., 2004; Montedoro, Servili, Baldioli, & Miniati, 1992). These compounds are mainly involved in the bitter taste of oil (Caponio et al., 2001; De Stefano, Piacquadio, Servili, Di Giovacchino, & Sciancalepore, 1999; Kiritsakis, 1998), confirming the role of each individual phenolic compound and not of total polyphenols in perceived bitterness of oil.

Favati, Caporale, Monteleone, and Bertuccioli (1995) found important differences in bitterness and astringency among oils obtained from different cultivars that were not always characterised by a high total polar phenol content. For example, oils obtained from unripe *Koroneiki* olives were more bitter and more astringent than the respective oils from *Coratina*, though in the latter the total phenol content was twice as high. As reported by Servili et al. (2009), the organoleptic properties of EVOO are largely affected by their phenolic composition. In fact, it is assumed that the stimuli responsible for bitterness in virgin olive oils are tyrosol, hydroxytyrosol, and their derivatives. As reported by Garcia et al. (2001), for some olive varieties a good correlation between oil bitterness and content of hydroxytyrosol secoiridoid derivatives has been found.

In a study performed by Mateos et al. (2004) the bitterness of the phenolic fraction of virgin olive oil was evaluated. None of the simple components of the phenolic fraction of olive oil, such as hydroxytyrosol, tyrosol, vanillic acid, vanillin, *p*-coumaric acid, ferulic acid, cinnamic acid, has been found to be responsible for bitter taste. On the other hand, the secoiridoid derivatives have shown a high intensity of this attribute, since all panellists detected it at the initial concentration (0.05 mM). EVOO, containing significant amounts of pinoresinol and 1-acetoxypinoresinol (0.15 mmol/kg) and flavones (0.05 mmol/kg) and a very low concentration of secoiridoid derivatives (0.04 mmol/kg), did not have a bitter taste.

Taking into account these results, the use of total phenol concentration as bitter predictor is not recommended, in disagreement with Beltràn et al. (2007).

3.2.2. Predictive model based on K_{225} values

In order to build up a perceived bitterness predictive model, Gutiérrez Rosales et al. (1992) proposed the evaluation of oil bitter taste by measuring the absorbance at 225 nm of polar extract. This "bitter index" or K_{225} value has been considered and discussed in several studies (Beltràn et al., 2007; Inarejos-Garcia et al., 2009; Mateos et al., 2004). Gutiérrez Rosales et al. (1992) have reported that a K_{225} value $\geqslant$0.360 correspond to quite bitter oils, which are refused by consumers. This bitter index was closely related

Table 3
Correlation between K_{225} value and bitterness intensity (B.I.) for each oil group studied.

Cultivar	Bitterness intensity		
	Equation	R^2	p
Coratina	B.I. = 14.247 $*$ K_{225} − 0.585	0.97	0.001
Leccino	B.I. = 14.514 $*$ K_{225} − 0.732	0.93	0.001
Maiatica	B.I. = 6.670 $*$ K_{225} − 0.412	0.97	0.001
Ogliarola	B.I. = 14. 727 $*$ K_{225} − 0.782	0.96	0.001
Blend	B.I. = 12.311 $*$ K_{225} − 0.272	0.89	0.001

with the intensity of bitter taste evaluated by an analytical panel of tasters with a correlation coefficient of $r = 0.914$ (Gutiérrez Rosales et al., 1992).

In this study, the use of K_{225} value as bitterness predictor has been considered, taking into account the relative simplicity of this analytical procedure, compared with other methods recently proposed, such as fluorimetry (Inarejos-Garcia et al., 2009). Initially, in order to test the versatility of the chosen chemical parameter, the data were treated separately by cultivar and blend. For the purpose, seven samples for each cultivar (including the blend samples) were used. As reported in Table 3, five linear correlations were obtained by relating mean bitterness intensity ratings with mean K_{225} values. The regressions were statistically significant for all groups ($p \leqslant 0.001$; $r^2 \geqslant 0.89$). Moreover, the slopes for the equations obtained from the regression between sensory bitterness and K_{225} value were similar for all cultivars, ranging from a minimum of 12.31 (blend) to a maximum of 14.73 (Ogliarola), with the exception of oil samples from the cultivar Maiatica (slope = 6.67) (Table 3). The low value of the slope of Maiatica sample set is probably due to the fact that all oil samples were characterised by a low bitterness intensity.

With the same purpose, Mateos et al. (2004) working with a total of 46 oil samples divided in 6 cultivars, found a poor correlation between the perceived bitterness and the chemical bitterness index proposed by Gutiérrez Rosales et al. (1992). These results could depend on the linear scale utilised for the in sensory evaluation; in fact no significant correlations were observed for the varieties characterised either by very high (*Hojiblanca*) or very low (*Arbequina*) bitterness intensity. The use of a non-structured scale, as suggested by the official method (EC Reg. 796/02) in sensory analysis and used in our study, may probably allow a better correlation between chemical and sensory bitterness evaluation, besides a better discrimination of oils from different cultivars in terms of perceived bitterness.

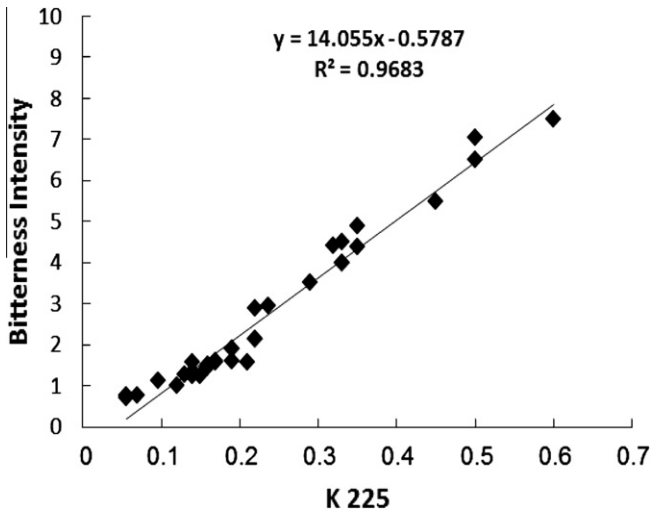

Fig. 2. Relationship between K_{255} value and perceived bitterness intensity.

Table 4
Predictive model validation. Comparison between predicted and measured bitterness intensity (Student t-test) by either the proposed prediction equation or that proposed by Gutiérrez Rosales et al. (1992).

Samples	Bitterness intensity		
	Predicted	Measured	Level of significance ($p < 0.05$)
1	0.74 ± 0.35*	0.98 ± 0.16	n.s.
2	1.28 ± 0.35	0.98 ± 0.38	n.s.
3	2.10 ± 0.35	2.06 ± 0.19	n.s.
4	2.79 ± 0.35	3.59 ± 0.15	0.03
5	3.08 ± 0.35	3.50 ± 0.60	0.01
6	3.50 ± 0.35	3.38 ± 0.15	n.s.
7	3.78 ± 0.35	4.50 ± 0.16	0.03
8	3.91 ± 0.35	3.50 ± 0.19	n.s.
9	4.34 ± 0.35	4.43 ± 0.32	n.s.
10	5.45 ± 0.35	4.92 ± 0.16	n.s.

n.s.: Not significant.
* Standard error.

Taking into account the abovementioned considerations, we tried to build up a general olive oil bitterness predictive model by relating mean intensity bitterness ratings to the mean K_{225} values of all samples. The linear regression obtained was significant; in particular, the r^2 coefficient associated with the predictive model built by using K_{225} values was higher (0.97) (Fig. 2) than the correlation coefficient obtained using total phenol concentration as bitterness predictor (0.42) (Fig. 1). Moreover, the standard error for the predictive model build up by K_{225} values was lower (0.35) than the error of the predictive model built up using total phenol content (1.21). The goodness of fit was also estimated, with residual values never higher than 0.40 (as absolute value), with respect to residual values of 2.33 (as absolute value) assessed for the regression obtained by using the total phenol content as bitterness predictor.

On the basis of the results obtained, the oil bitterness intensity could be predicted by using the K_{225} values of oil samples in the following equation: Bitterness Intensity = 14.055 $*$ K_{225} − 0.5787 (Fig. 2).

The results of this study encourage the use of the K_{225} value for estimating the bitterness of EVOO, independently of the cultivar considered.

3.3. Predictive model validation

In order to allow a practical application and to verify the reliability of the results obtained, the proposed model was validated using unknown EVOO samples. A test sample set was then prepared, consisting of 10 oil samples, two for each cultivar/blend utilised in the study. Results from the one-way analysis of variance carried out on the bitterness ratings of the oil test set showed a significant effect of the factor "sample" ($p \leqslant 0.05$), thus the number of samples included in the test set was assumed to be sufficient for validating the proposed predictive model (Table 4).

The predictive capacity of the model was assessed by comparing measured and predicted bitterness intensity in the test set samples. The data showed that the predicted bitterness intensity scores were not significantly different (Student t-test at $p < 0.05$) from the measured mean scores, except for three samples (4, 5 and 7; Table 4). In this case the predictive model underestimated the bitterness intensity; however it should be pointed out that in all three cases the difference between the predicted and the measured mean value was less than 0.8. Thus, the risk of a limited underestimation of the predicted bitterness intensity was reasonably assumed not to affect the reliability of the predictive model.

The results of this study confirm the suitability of the proposed method in predicting the bitterness intensity of EVOO. The simplicity of the analytical method used and the excellent results of the validation tests of the predictive model may allow a specific use of the proposed method in on-line monitoring of oil quality in terms of perceived bitterness.

4. Conclusions

In this study, the possibility of estimating the perceived bitterness intensity in different EVOO by using chemical indices, such as the total phenol content and the compounds measured spectrophotometrically at 225 nm (K_{225} value) as bitterness predictors, has been evaluated. In order to improve the predictive capacity of the model, sensory analysis has been performed using a non-structured linear scale, which has a higher discrimination power than five-point category scale, allowing a better differentiation among oil samples. A bitterness predictive model has been built; furthermore, the predictive capacity of the model has been validated, by comparing predicted and measured bitterness intensities in a test set of unknown oil samples. The results indicated that the oil bitterness intensity could be satisfactorily predicted by using the K_{225} values of oil samples in the following equation: Bitterness intensity = $14.055 * K_{225} - 0.5787$. The proposed predictive model appears as a complementary tool in the characterisation of EVOO samples in the routine analysis on the basis of perceived bitterness intensity.

Although the predictive model may allow a good discrimination of the oils based on their bitterness intensity, this model could be improved by the implementation of hedonic tests for sensory evaluation, in order to identify the maximum level of acceptability of oil bitterness for consumers.

Acknowledgements

The authors would like to thank Luca Pappalardo for his skilful technical assistance. The authors express their gratitude to all extra virgin olive oil-makers for supplying the samples of precisely known origin.

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