JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Sensory Properties of Virgin Olive Oil Polyphenols: Identification of Deacetoxy-ligstroside Aglycon as a Key Contributor to Pungency

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Polyphenols are an important functional minor component of virgin olive oils that are responsible for the key sensory characteristics of bitterness, pungency, and astringency. Polyphenols were isolated from virgin olive oils by using liquid/liquid extraction and then separated by using reverse phase HPLC followed by fraction collection. The sensory qualities of the isolated polyphenols were evaluated, and almost all fractions containing polyphenols were described as bitter and astringent. However, the fraction containing deacetoxy-ligstroside aglycon produced a strong burning pungent sensation at the back of the throat. In contrast, the fraction containing the analogous deacetoxy-oleuropein aglycon, at an equivalent concentration, produced only a slight burning/numbing sensation, which was perceived more on the tongue. No other polyphenol fractions from the analyzed oils produced the intense burning sensation; thus, deacetoxy-ligstroside aglycon is the polyphenol responsible for the majority of the burning pungent sensation found in pungent extra virgin olive oils.

KEYWORDS: Bitterness; astringency; pungency; burning sensation; virgin olive oil; deacetoxy-ligstroside aglycon; deacetoxy-oleuropein aglycon

INTRODUCTION

Polyphenols is a broad term used in the natural products literature to define substances that possess a benzene ring bearing one or more hydroxy groups, including functional derivatives. In the case of virgin olive oils, "polyphenol" mostly refers to hydrolysis products of oleuropein and ligstroside aglycons and related compounds. These compounds are responsible for the oxidative stability of virgin olive oils (*1*−*4*) and are associated with health benefits (*1*, *2*, *5*−*8*). Polyphenols also contribute to the organoleptic properties of virgin olive oils and are commonly described as bitter and astringent. Less commonly, polyphenols are associated with pungency, that is, peppery, burning, or hot sensations (*3*).

Polyphenols have very similar chemical structures, and there is a correlation between the total polyphenol concentration in olive oils and olive oil bitterness; thus, it is generally assumed that most polyphenols have similar bitterness thresholds. Although it is clearly established that polyphenols as a group are the main contributors to olive oil bitterness and astringency, the contribution that each individual polyphenol makes to the total bitterness is unclear. Furthermore, the relationship between polyphenols and olive oil astringency and pungency is also uncertain; for instance, it is not known if all bitter polyphenols are also astringent and pungent. Attempts have been made to correlate concentrations of individual polyphenols to panel test scores, but these have often had little success (*9*). A strong correlation between the concentration of secoiridoid derivatives of hydroxytyrosol and bitterness was reported, but only if one olive variety was used (*10*). In contrast, a strong correlation between a secoiridoid derivative of tyrosol and the bitter and pungent sensory attributes has also been reported (*11*).

Some researchers suggest that secoiridoid derivatives of hydroxytyrosol are the main contributors to olive oil bitterness (*2*, *10*, *13*), but the only situation in which this suggestion might be true is in oils in which secoiridoid derivatives of hydroxytyrosol are the main components, but this is not always the case, and secoiridoid derivatives of tyrosol may also contribute to bitterness.

To establish the organoleptic contributions that individual polyphenols make to an olive oil, it is important to either synthesize or isolate individual polyphenols for sensory analysis. Synthesis is currently not practical, so isolation procedures must be used.

Much literature is available on the development of methods for the analysis, isolation, and identification of polyphenols in olives and olive oils (*1*−*3*, *5*, *9*, *10*, *14*−*21*). Typically, these methods have been applied to monitoring differences between olive varieties and changes during ripening and oil processing. The isolation of polyphenols has been performed to assess their

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10.1021/jf026042j CCC: $25.00
Published on Web 01/25/2003

individual properties as antioxidants (*17*). However, the sensory properties of these isolated polyphenols have not been investigated.

In an attempt to investigate the sensory properties of individual polyphenols, Gutierrez (*9*) and co-workers fractionated polyphenols in an unspecified extra virgin olive oil by using preparative HPLC and found four major peaks. The material in these peaks was resuspended in water and presented to a panelist for tasting. The four components were described as hot, slightly bitter, strongly bitter, and slightly bitter, respectively. The isolated peaks were not identified; however, when the bitterest fraction was analyzed by using analytical HPLC, the fraction consisted of at least three compounds.

It is known that oleuropein is the bitter principle found in olives; however, it is not found at significant concentrations in olive oils, but oleuropein aglycon and isomers of oleuropein aglycon are. An unknown isomer (or isomers) of oleuropein aglycon was prepared by β-glucosidase hydrolysis of oleuropein isolated from olives (*22*). This was evaluated and found to be bitter with a threshold of ~50 μg (*22*). Using the same evaluation technique, no bitterness was observed for hydroxytyrosol or elenolic acid. No other polyphenols were evaluated. Some pungency was also associated with oleuropein aglycon by some of the panelists.

Assessment of bitterness, astringency, and pungency is difficult. Problems arise due to the lingering of the sensations on the palate, desensitization after exposure to a strong sample, and difficulty in differentiating between sensations such as bitterness and pungency. Recently, a procedure called taste dilution analysis (TDA) was reported (*23*, *24*). TDA is analogous to aroma extract dilution analysis (AEDA). Bitterness is assessed by preparing serial dilutions of samples in water and then tasting in order of increasing concentration until the concentration is found at which the diluted sample can be differentiated from water as judged in a triangle test.

In this investigation we used reverse phase HPLC to isolate polyphenols from virgin olive oils and applied sensory evaluation (TDA) to these samples to characterize the polyphenol that is responsible for the majority of the pungency (burning sensation) found in many olive oils.

MATERIALS AND METHODS

Materials. Spanish, Italian, Greek (unknown regions), and Andalusian (Spain) olive oils were obtained from industrial mills. Oils were milled using standard processing conditions. Tyrosol [2-(4-hydroxyphenyl)ethanol] was purchased from Aldrich. Hydroxytyrosol [2-(3,4-dihydroxyphenyl)ethanol] had previously been synthesized in our laboratory according to a literature method (*25*). All materials used were of food grade, apart from the hexane, used for the extraction step, and methanol used for one HPLC run.

High-Performance Liquid Chromatography (HPLC). The isocratic HPLC system consisted of an HPLC pump (Waters 510), an autosampler (Waters 717+), a UV absorbance detector (Waters Lambda Max 481), and a fraction collector (Pharmacia LKB-SuperFrac), and data were recorded and analyzed by using TurboChrom software and interface (Perkin-Elmer). The 250 × 4.6 mm i.d. analytical scale HPLC column was packed with 5 μm Phenomenex Luna (phenyl-hexyl) stationary phase. A SecurityGuard (Phenomenex) guard cartridge was placed upstream of the analytical column. The column and guard cartridge were maintained at 35 °C. The mobile phase consisted of 30% food grade ethanol (96.4%, Royal Nedalco B.V.) and 70% acidified Milli-Q water (2% acetic acid) at a flow rate of 1 mL/min. The eluting compounds were detected at 280 nm.

This HPLC configuration was used in either an analytical mode or a semipreparative mode. In the analytical mode the injection volume was between 10 and 100 μL. In the semipreparative mode the injection volume was 1 mL and multiple injections were made. In both cases the same size HPLC column was used and no problems were encountered due to column overload.

Mass Spectrometry. An Agilent 1100 MSD was used with electrospray ionization in the negative ion mode with a fragmentor voltage of 70 V. Samples were directly injected into the MS by a syringe pump with a flow rate of 50 μL/min. The scan range was between 60 and 700 Da.

Nuclear Magnetic Resonance (NMR) Spectroscopy. Carbon and proton NMR spectra were recorded on a Bruker DRX600 spectrometer, operating at 600.13 and 150.9 MHz for ^{1}H and ^{13}C, respectively. Spectra were collected at 298 K.

Isolation of Polyphenols. The extraction procedure was based on the procedure reported by Montedoro and co-workers (*26*). Olive oil (200 g) was dissolved in hexane (400 mL) and extracted once with 400 mL of ethanol/water (60:40) for 2 min. The aqueous phase was collected and washed with another 400 mL of hexane. The ethanol/water phase was separated from the hexane phase, and the hexane phase was discarded. The ethanol/water extract was rotary evaporated (water bath at 40 °C) to a residue. The residue was redissolved in 25 mL of HPLC mobile phase [2% aqueous acetic acid/ethanol (70:30)], and this solution was filtered through a 0.45 μm syringe filter.

The olive oil polyphenol extract was fractionated by using HPLC. Fractions from 20 HPLC runs (injection volume = 1 mL, two fractions per minute from 2 to 62 min) were collected and combined in polyethylene test tubes (12 mL). The content of every second fraction was determined by using HPLC. On the basis of these results, fractions were pooled into groups. No fractions were discarded.

To separate the polyphenols from the HPLC mobile phase components (ethanol and acetic acid), solid phase extraction (SPE) was used. Reverse phase SPE columns (Bond Elut C18, 500 mg, 10 mL) were obtained from Phenomenex. Samples were processed in batches containing up to 12 samples by using a 12 port vacuum manifold. SPE columns were primed by rinsing them with 10 mL of food grade ethanol followed by 10 mL of water. A sample was loaded onto an SPE cartridge by dilution of the sample four times in water and then passing the diluted material through the SPE cartridge (~3 mL/min). The SPE cartridge was then rinsed with 10 mL of water to remove residual acetic acid. The sample was then eluted from the SPE cartridge by using 5 mL of ethanol. Water (~20 mL) was added to the ethanol solution, and then rotary evaporation was performed until all of the ethanol had evaporated. The solution was made to a final volume of 20 mL with water and filtered through a 0.45 μm syringe filter.

The resulting polyphenol solution was used for the sensory evaluation, and each polyphenol solution was partially characterized by using HPLC (detection at 280 nm) and mass spectrometry (direct injection). Polyphenols were *tentatively* identified on the basis of HPLC elution order, molecular weights, and literature data.

Sensory Evaluation. The taste dilution factors for fractions collected between 12 and 62 min during the HPLC fractionation of Andalusian olive oil polyphenols were determined. From the taste dilution factors, taste thresholds were estimated for tentatively identified compounds. Furthermore, the taste thresholds of other polyphenols, not found in Andalusian olive oil at sufficient concentrations, namely, deacetoxyoleuropein aglycon and the polyphenol corresponding to peak 7a, were also estimated. These polyphenols were isolated from a Greek olive oil. Taste thresholds were estimated by using TDA as described in the literature (*23*, *24*) and further detailed below.

Four panelists (one male and three females) were selected on the basis of their ability to successfully complete a basic taste test and their performance on a bitterness-ranking test.

For each polyphenol sample a series of five dilution steps was prepared, from 16× dilution to full strength. Each of these diluted samples was evaluated in a triangle test. In each triangle test two samples of water and the sample of diluted compound were labeled with a randomly selected three-digit number and presented to the panel in a random order. The panelists, while wearing a nose clamp, were requested to dispense ~0.7 mL of solution onto a spoon and taste the solution. Panelists were asked to identify which sample was not water, on the basis of any of the five tastes or any trigeminal sensation. The possibility of picking the sample out due to an aroma aspect was

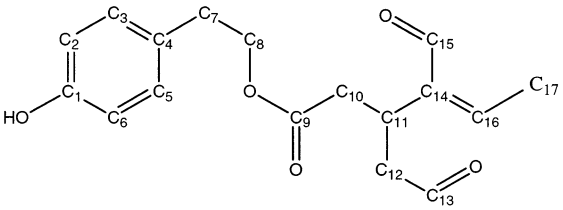

Figure 1. Structure of deacetoxy-ligstroside aglycon. Deacetoxy-oleuropein aglycon has a hydroxy group at C2 (C6).

eliminated by the use of the nose clip and the fact that most fractions contained very little aroma. Samples were evaluated at room temperature, and because the samples were clear and colorless, no special lighting was used.

If a panelist got a triangle test correct but then failed on the next triangle test, at a higher concentration, then the former triangle test result was considered only to be a correct guess and ignored. Only when a panelist got at least two triangle tests in a row correct was that individual considered to have passed the taste threshold. The taste dilution thresholds reported by each panelist were averaged. Polyphenol samples were evaluated in random order. In an open discussion, during which panelists were able to taste polyphenol samples at will, a list of descriptors for the polyphenol samples was generated.

Isolation, Purification, and Identification of the Pungent (Burning) Polyphenol. A sample of the polyphenol that was described as producing a burning sensation was prepared from a Greek olive oil and concentrated to a volume of 2 mL. This material was subjected to a second purification step on a different HPLC system. Two duplicate injections (1 mL injection volume) were made on a 250 × 4.6 mm i.d. Inertsil ODS-2 column with a gradient of methanol/water at ambient temperature, as reported previously (*5*). Fractions were collected at a frequency of two fractions per minute, and the fractions from the two HPLC separations were combined. The fraction containing the highest concentration of the pungent polyphenol was selected. The acetic acid and methanol were separated from the polyphenol by using SPE, and the polyphenol was redissolved in water and evaluated by the panel. The compound responsible for the pungent (burning) sensation was identified as deacetoxy-ligstroside aglycon (**Figure 1**) by using NMR and MS.

MS (ES^-), *m/z* 303 (100, [M − 1]), 607 (57, [M_2 − 1]), 361 (20), 349 (20), 285 (21), 97 (10); ^{1}H NMR ($CDCl_3$, 600.13 MHz) δ 2.08 [d, 3H, J = 7.1 Hz, H−C(17)], 2.62 [dd, 1H, J = 15.6, 6.6 Hz, H_A−C(10)], 2.68 [dd, 1H, J = 15.6, 8.0 Hz, H_B−C(10)], 2.74 [dd, 1H, J = 18.3, 5.5 Hz, H_A−C(7)], 2.84 [t, 2H, J = 7.0 Hz, H−C(7)], 2.99 [dd, 1H, J = 18.3, 8.5 Hz, H_B−C(7)], 3.62 [m, 1H, H−C(11)], 4.22 (m, 2H), 6.63 [q, 1H, J = 7.1 Hz, H−C(16)], 6.76 [d, 2H, J = 8.4 Hz, H-C(2,6)], 7.07 [d, 2H, J = 8.4 Hz, H−C(3,5)], 9.25, 9.64 [br s, 1H, H−C(13), H−C(15)]; ^{13}C NMR ($CDCl_3$, 150.9 MHz) δ 15.25 [CH_3, C(17)], 26.97 [CH, C(11)], 34.24 [CH_2, C(7)], 36.82 [CH_2, C(10)], 46.20 [CH_2, C(12)], 64.72 [CH_2, C(8)], 115.37 [CH, C(2,6)], 130 [C, C(4)], 130.14 [CH, C(3,5)], 143.39 [C, C(14)], 153.82 [CH, C(16)], 154 [C, C(1)], 171.99 [CO, C(9)], 195.09, 200.25 [CH, C(13), C(15)].

RESULTS AND DISCUSSION

An analytical scale HPLC column, which was subjected to large injection volumes (1 mL) and high polyphenol concentrations, was used for the isolation of polyphenols. This size column provided more than adequate sample quantities and polyphenol separation, with no evidence to suggest that the column had been overloaded. The fractions from the HPLC were pooled into groups, the acetic acid and ethanol removed by using the SPE procedure, and the contents of each sample determined. Most polyphenol samples contained only one or two major peaks (**Figure 2**). Higher purity samples could have been obtained if some fractions were discarded rather than pooling them in with the group, but we wanted to ensure that all material was evaluated so that all components would be evaluated. These samples were found to be reasonably stable. After storage of

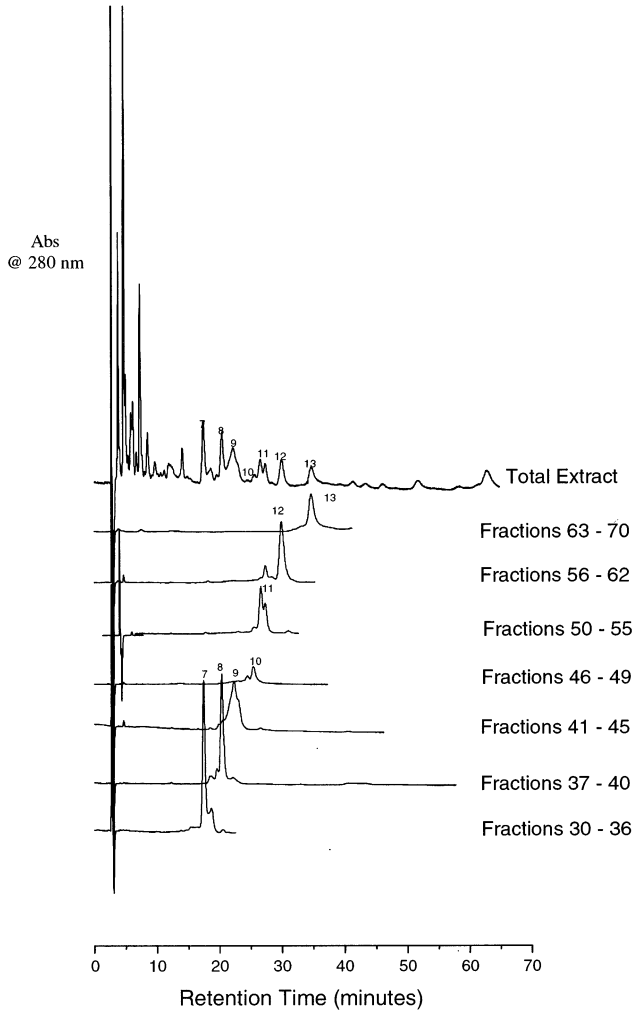

Figure 2. HPLC chromatograms of polyphenol fractions isolated from an Andalusian olive oil and the HPLC chromatogram of the Andalusian olive oil.

the samples for 1 month at 4 °C, samples were analyzed by using HPLC and there was no evidence of hydrolysis or oxidation. All panel evaluations were performed within 1 week of sample preparation.

Analysis of the phenolic extracts from a large variety of olive oils in our laboratory and by other researchers (*5*, *14*, *15*, *18*, *19*) has shown that there are about 12 major peaks in the reverse phase HPLC chromatogram. The proportions and intensities of these peaks differ between olive oil varieties. For this work, we analyzed Italian, Greek, Spanish (unknown regions), and Andalusian (Spain) olive oils by using the HPLC technique that we also used for fractionation. We also found approximately 12 major peaks as well as some minor peaks. The differences between olive oil varieties were evident only as differences in the ratios and intensities of these peaks. The polyphenols evaluated by the panelists in this work were therefore representative of almost all of the polyphenols found in a wide range of extra virgin olive oils.

On the basis of MS data, elution order, and literature data, *tentative* identification of the main polyphenols was made. From the MS fragmentation pattern it could be ascertained if the compound was a derivative of tyrosol or hydroxytyrosol. Further identification was considered useful only for those polyphenols with exceptional sensory properties.

Taste Dilution Profile of an Andalusian (Spain) Olive Oil. TDA was performed on fractions obtained by separation of the

Table 1. Sensory Qualities, Taste Thresholds, and Tentative Identifications of Components Isolated from Extra Virgin Olive Oils

peak	main component (tentative identification[a])	sensory qualities	estimated taste threshold (mM)
1[a]	hydroxytyrosol[b]	[b]	[b]
2	tyrosol	sticking astringency, not bitter	4.4–18
5	deacetoxy-oleuropein aglycon (dialdehydic form)	astringent, bitter, burning/stinging/numbing mostly on tongue (not nearly as strong as that experienced with peak 9)	0.4–1.6
7	derivative of oleuropein aglycon	bitter, sour, astringent, sweet, cooling, peppery (tingling tongue)	0.2–0.8
7a	not identified	bitter, astringent (dry teeth)	0.2–0.8
8	derivative of oleuropein aglycon	bitter, astringent, bit burning	0.1–0.4
9	deacetoxy-ligstroside aglycon (dialdehydic form)	strong burning mostly at the back of throat, slightly bitter, astringent	0.4–1.6
10	isomer of ligstroside aglycon	astringent, bit burning, bitter	0.05–0.2
11	isomer of ligstroside aglycon	dry mouth, not bitter	0.4–1.6
12	derivative of oleuropein aglycon	bitter, astringent, salt	0.1–0.4
13	isomer of oleuropein aglycon	very bitter, very astringent	0.05–0.2
	hydrophobic polyphenols (fractions 70–90)	strong bitter, astringent	
	very hydrophobic polyphenols (fractions 90–120)	astringent, bitter, bit sour, bit burning, salt	

[a] All identifications are tentative except for peak 1 (identified in comparison with hydroxytyrosol standard), peak 2 (identified in comparison with tyrosol standard), and peak 9 (where NMR, MS, and other experiments were used). [b] Although synthetic hydroxytyrosol was available, it was not evaluated by the panel because the sample was not of sufficient purity to be safely evaluated.

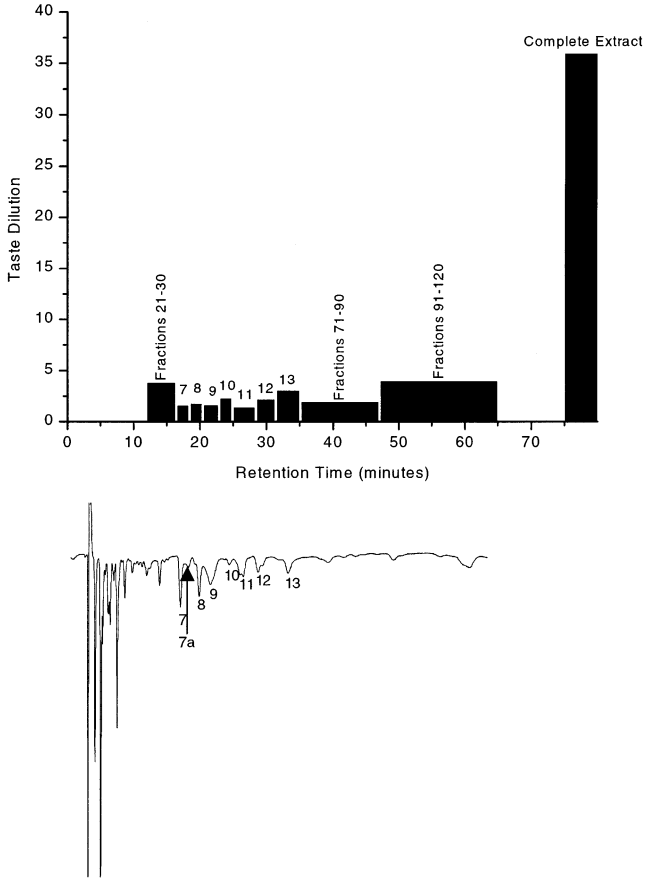

Figure 3. Taste dilution profile of an Andalusian olive oil.

polyphenols extracted from an Andalusian (Spain) olive oil. Most fractions had similar taste dilution factors (**Figure 3**).

In one experiment, the taste dilution factor of an olive oil polyphenol extract was determined before HPLC fractionation. The extract was fractionated, and all material eluting from the HPLC between 2 and 62 min was collected and combined. The polyphenols in this sample were separated from the mobile phase, and then the taste dilution factor was determined. There was no significant difference in the taste dilution factors before and after the fractionation procedure. Thus, taste components in the original extract are not lost during the fractionation procedure by, for example, irreversible absorption to the column or hydrolysis.

The taste thresholds of the tentatively identified compounds were estimated on the basis of assumptions that each fraction contained only one compound and that peak area (for absorbance at 280 nm) is proportional to concentration with no difference in response factors among the different polyphenols (**Table 1**). This was also done for a sample of synthetic tyrosol, a sample containing deacetoxy-oleuropein aglycon, and a sample containing the polyphenol corresponding to peak 7a, isolated from a Greek olive oil (**Table 1**). Although the purity of the compounds isolated is not 100% and concentrations were only estimated, these results suggest that the taste thresholds for different polyphenols (except tyrosol and hydroxytyrosol) are similar, which is consistent with their similar structures and the good correlation between total polyphenol content and bitterness. Furthermore, and more importantly, it should be noted that bitterness is spread throughout the chromatogram, so it is highly unlikely that olive oil bitterness is due to just one or maybe two bitter polyphenols but rather is due to the total contribution of a number of different polyphenols. Further research is required to develop methods that deliver 100% pure polyphenols in a food grade format in sufficient quantities for panel evaluation.

Panelists reported the sensory qualities of each polyphenol fraction (**Table 1**). Almost all fractions were bitter and astringent. Although it seems that the amount of astringency relative to bitterness, and in some cases the type of astringency, varies from fraction to fraction, it is very difficult for panelists to describe this in a quantitative manner.

Taste dilution is not specific to bitterness. Panelists are asked only to select the sample in the triangle test that is not water (with aroma aspects eliminated by the nose clamp). Therefore, if two fractions have the same taste dilution factor, one may actually be significantly more bitter than the other, the other having a taste dilution factor more attributable to either astringency or burning sensation. The development of more reliable quantitative techniques to determine bitterness thresholds of polyphenols when other sensory qualities (astringency and pungency) are present and vice versa is an area that requires further research.

Unlike most fractions, which were just bitter and astringent, the polyphenol sample corresponding to peak 9 proved to be

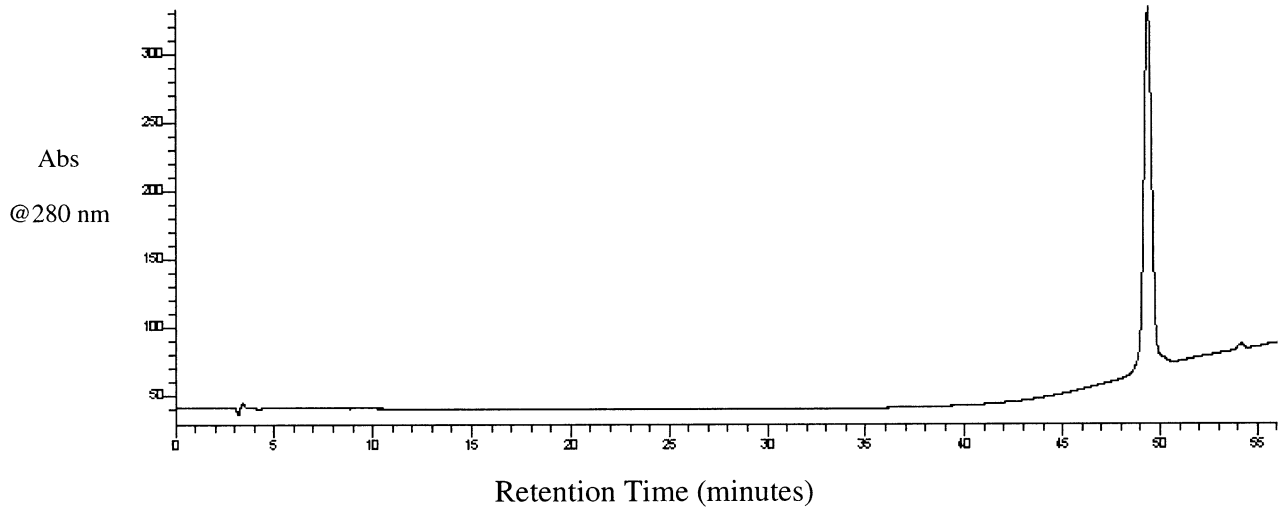

Figure 4. HPLC chromatogram of purified deacetoxy-ligstroside aglycon.

particularly interesting. The sample containing peak 9 was described as causing a strong burning or prickling sensation that is predominantly felt at the back of the throat, although at high concentrations it is also felt slightly on the tongue. The sensation is similar to that experienced when tasting capsaicin, the hot component of chilly peppers. Although some of the panelists describe this sensation for some of the other samples, it was only for peak 9 that panelists unanimously perceived the burning sensation, and all panelists agreed that the burning sensation was much greater in the peak 9 sample than in any of the others. The burning sensation perceived by some panelists in the fractions corresponding to peaks 8 and 10 might be due to contamination with peak 9.

Identification of the Pungent (Burning) Polyphenol. Of all the polyphenol samples tasted by the panelists, the sample corresponding to peak 9 was notably different because it produced a strong burning sensation at the back of the throat. Analysis of this sample by MS showed that it contained predominantly one component with a molecular weight of 304. On the basis of the literature, it was concluded that the predominant polyphenol in this sample is 4-hexenoic acid, 4-formyl-3-(2-oxoethyl)-2-(4-hydroxyphenyl) ethyl ester, also known as the deacetoxy form of ligstroside aglycon (**Figure 1**), which has been commonly reported as an important component of many extra virgin olive oils (*18*). This identity was confirmed by using ^{1}H and ^{13}C NMR. Both the ^{1}H and ^{13}C NMR spectra of this sample corresponded to the literature data (*18*) for this compound. Furthermore, base hydrolysis of the burning polyphenol sample gives rise exclusively to tyrosol, the expected hydrolysis product (see below). Although the burning sensation and deacetoxy-ligstroside aglycon occur in the same fraction, it does not necessarily mean that deacetoxy-ligstroside aglycon is the source of the burning sensation, even if it is implicated. Samples of deacetoxy-ligstroside aglycon were isolated not only from Andalusian (Spain) olive oil but also from three other different olive oils [a Spanish olive oil (unknown region), an olive oil enriched in polyphenols, and a Greek olive oil], and in all cases the isolated material produced the burning sensation. Thus, either deacetoxy-ligstroside aglycon or a coeluting compound is causing the burning sensation. More experiments were done to demonstrate that deacetoxy-ligstroside aglycon is responsible for the burning sensation associated with peak 9. One approach to show that deacetoxy-ligstroside aglycon is responsible for the burning sensation is to synthesize and taste it, but this was not considered because the synthesis is extremely challenging.

Polyphenol samples are susceptible to base hydrolysis. Thus, to determine if a polyphenol is the source of the burning sensation, the polyphenol sample containing the burning sensation was made slightly alkaline by adding a small quantity of sodium bicarbonate solution. The solution was evaluated immediately after the base had been added and found to still produce the burning sensation; that is, the bicarbonate did not mask the burning sensation. After alkaline hydrolysis overnight, the solution was evaluated and it clearly no longer had any burning sensation, although it was astringent and slightly bitter. Analysis by HPLC showed the complete absence of deacetoxy-ligstroside aglycon, but the expected hydrolysis product, tyrosol, was present.

To further demonstrate that impurities coeluting with deacetoxy-ligstroside aglycon do not cause the burning sensation, a high-purity sample of the deacetoxy-ligstroside aglycon was prepared by using a completely different HPLC system. The analytical HPLC (using an ODS column and gradient method) chromatogram of the purified deacetoxy-ligstroside aglycon is shown in **Figure 4**. When the purified deacetoxy-ligstroside aglycon was evaluated, it was found to still have the characteristic burning sensation. It is unlikely that a compound that coelutes with deacetoxy-ligstroside aglycon, when using the phenyl-hexyl column, would also coelute with deacetoxy-ligstroside aglycon) when using the ODS column, because the selectivities of the two columns are different. Thus, it is unlikely that the burning sensation is due to a trace impurity coeluting with the deacetoxy-ligstroside aglycon.

To assess the total contribution that peak 9 makes to the total pungency of a pungent olive oil, we prepared an olive oil from which peak 9 was selectively removed. This was done by fractionating a pungent olive oil (using the methods described in this paper), combining all of the fractions except that corresponding to peak 9, and then resuspending the sample in a refined olive oil. Comparison of the oil lacking peak 9 with the oil containing peak 9, by a panel evaluation, proved to be difficult, but we estimate that the removal of peak 9 causes at least a 60% reduction in olive oil pungency.

These results have implications for controlling the pungency in olive oils. By carefully controlling parameters such as olive variety, harvest time, and processing conditions, oils can be created with different amounts of deacetoxy-ligstroside aglycon and hence different levels of pungency.

Until now the relationship between polyphenols and olive oil pungency has been unclear. In this research we assessed the contribution of polyphenol fractions from extra virgin olive oils to olive oil bitterness, astringency, and pungency. When panelists were asked to describe the sensory properties of each polyphenol fraction, most fractions were described as bitter and astringent. However, one polyphenol fraction was significantly different from the others because it was described as producing a strong pungent (burning) sensation at the back of the throat. Further purification, omission analysis, and correlation and hydrolysis studies conclusively showed that deacetoxy-ligstroside aglycon is the key source of the burning sensation found in many olive oils. In contrast, deacetoxy-oleuropein aglycon (the hydroxytyrosol analogue), tasted at an equivalent concentration, produced very little burning sensation. This is a clear example of different sensory properties of a secoiridoid derivative of hydroxytyrosol and tyrosol. Therefore, the practice of correlating sensory scores to only secoiridoid derivatives of hydroxytyrosol can be misleading.

ACKNOWLEDGMENT

We thank Are Pripp, Bep Knoops, Jan van Buuren, Han van Brouwershaven, and Max Batenburg for their assistance.

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Received for review October 16, 2002. Revised manuscript received December 17, 2002. Accepted December 17, 2002.

JF026042J