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Relationship between Virgin Olive Oil Phenolic Compounds and Acrylamide Formation in Fried Crisps

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In this paper the relationship between virgin olive oil (VOO) phenol compounds and the formation of acrylamide in potato crisps was investigated. The phenol compositions of 20 VOO samples were screened by LC-MS, and 4 oils, characterized by different phenol compound patterns, were selected for frying experiments. Slices of potatoes were fried at 180 °C for 5, 10, and 15 min, and acrylamide content was determined by LC-MS. Results demonstrated that VOO phenolic compounds are not degraded during frying, and crisp color was not significantly different among the four VOOs. Acrylamide concentration in crisps increased during frying time, but the formation was faster in the oil having the lowest concentration of phenolic compounds. Moreover, the VOO having the highest concentration of ortho-diphenolic compounds is able to efficiently inhibit acrylamide formation in crisps from mild to moderate frying conditions. It was concluded that the use of ortho-diphenolic-rich VOOs can be proposed as a reliable mitigation strategy to reduce acrylamide formation in domestic deep-frying.

KEYWORDS: Virgin olive oil; acrylamide; frying; potato crisps; Maillard reaction; phenol compounds

INTRODUCTION

Acrylamide has been classified as a probable carcinogen by the International Agency for Research on Cancer (*1*). It is formed through Maillard reaction (MR) in many starch-rich foods (*2*), and particularly high levels of acrylamide have been found in processed foods such as potato crisps, potato chips, crisp bread, bakery products, breakfast cereal, and coffee (*3*).

It has been claimed that dietary acrylamide does not constitute any risk to human health (*4*); however, considering the potential health hazard of this molecule and its wide distribution in the Western diet, numerous studies have been undertaken to provide reliable mitigation strategies to minimize its levels in processed products (*5, 6*).

Acrylamide in food is mainly formed by a reaction between asparagine and reducing sugars (*7–10*). The mechanisms of acrylamide formation were deeply investigated (*7, 11*), and the significant role of 3-aminopropionamide as reaction intermediate was elucidated (*12*).

Potato products, such as crisps and French fries, are among the major contributors to the acrylamide daily intake, especially for children and teens (*13*). In the past 5 years, many strategies aiming to reduce acrylamide formation in potato products have been proposed (*14*). The selection of potato varieties having a low concentration of free carbohydrates and the use of appropriate storage conditions are of pivotal importance (*6*). Many studies demonstrated that it is possible to define time–temperature processing conditions which guarantee low acrylamide concentration and the retention of sensorial properties in terms of color, flavor, and starch gelatinization (*15, 16*).

Different mitigation strategies have been also proposed: some of them are based on the addition of amino acid to potatoes before processing. In potato crisps, both glycine and, to a lesser extent, glutamine have a reducing effect on the content of acrylamide. The reason for the effect is not clear; glycine may compete effectively with asparagine in the Maillard reaction, or glycine may react with acrylamide (*17*).

The outcomes of these studies were used by the European food and drink federation (CIAA) to construct the acrylamide toolbox that for potato products recommends the reduction of free carbohydrate concentration in raw material and the adoption of mild frying conditions. Also thanks to this tool, in the past few years the acrylamide concentration has been significantly reduced in industrial products, but for potato products, domestic preparation remains a relevant source of acrylamide production (*18, 19*).

The addition of antioxidants to potatoes was frequently proposed to prevent acrylamide formation: acrylamide concentration was reduced by up to 50% when a spice mix was added to potato slices before frying and a powder spice mix was added to the slices after frying (*20*). Biedermann and co-workers (*6*)

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showed a weak inhibition effect upon addition of ascorbic acid to a potato-based model. The ability of ferulic acid to inhibit acrylamide formation was attributed to its capability to react with acrylamide precursors or intermediates in the chemical process of its generation (*21*). A recent paper indicated that the addition of antioxidant bamboo leaves effectively reduces the amounts of acrylamide in potato crisps and French fries (*22*).

On the other hand, studies about the effects on acrylamide formation of the antioxidant compounds present in the oil were scarce. Becalski et al. (*7*) found that acrylamide could be reduced when adding rosemary herb to the oil used for frying potato slices. It should be noted, however, that these findings could not be confirmed by others (*23*).

However, it is possible that the minor oil components can influence acrylamide formation during the exposure of the potatoes at high temperatures. Virgin olive oil (VOO) has a peculiar set of polar phenolic compounds, the secoiridoid derivatives, that exert a bewildering array of physiological and technological properties (*24*). These compounds are located at the interface between oil and the polar phase where, according to the so-called "polar paradox" (*25*), they can create a reducing environment that is very effective in protecting the oil from oxidation. Interestingly, the capability of VOO phenolic compounds to reduce the formation of MR products during frying has already been demonstrated: a reduction of up to 60% of the formation of the different heterocyclic amines (HAs) in fried burgers and in model system depending on the VOO phenolic compound concentration was found (*26, 27*). Data of these works demonstrated that the oils having high concentrations of oleuropein derivatives, that is, the ortho-diphenolic compounds, are much more efficient in this action (*26, 27*).

The aim of this paper was to verify if VOO phenol compounds present at the interface between oil and potato surface, where most of the acrylamide is formed, can affect its final concentration. Therefore, the possibility of reducing the content of acrylamide in fried potatoes by using VOO rich in antioxidant during frying procedures was investigated. Four VOO samples, having different phenolic compound patterns, were selected, and potato crisps were fried in these oils, monitoring the amount of acrylamide formed over different times. Results demonstrated that, in mild frying conditions, the higher the concentration of dihydroxyphenol compounds present in the oil, the lower the concentration of acrylamide formed in crisps.

MATERIALS AND METHODS

VOO Samples. Twenty samples of VOO produced from different olive varieties in various regions of southern Italy (Sicily, Campania, Apulia) were collected and stored in filled containers at 16 °C in the dark before they were submitted to chemical analyses and frying trials. All samples showed values of free acidity, peroxide number, and spectrophotometric indices within the limits for extra VOOs as defined by EC Regulation (CE) 1989/2003 relative to the characteristics of olive oils, olive pomace oils, and related analytical methods.

Chemicals. Solvents used for HPLC analysis were purchased from Merck (Darmstadt, Germany). Acrylamide (>99.5% purity), potassium ferrocianide (Carrez 1), and zinc acetate (Carrez II) were obtained from Sigma-Aldrich (St. Louis, MO). [$^{13}C_3$]Acrylamide (isotopic purity = 99%) was from Cambridge Isotope Laboratories (Andover, MA). Oleuropein standard was purchased from Extrasynthese (Paris, France). Pinoresinol was from PhytoLab GmbH & Co. (Vestenbergsgreuth Germany). Silica SPE-ed cartridges of 500/3 mL were from Applied Separation. All samples were filtered using cellulose filters RC 25 mm 0.20 μm (Chemtek Analytical) and 2 mL syringes (Plastipak).

Antioxidant Capacity. The antioxidant activity was determined directly on diluted VOO using the ABTS method as previously described (*28*). The absorbance was measured at 734 nm using a spectrophotometer (UV–vis Shimadzu 2100) equipped with a temperature control unit (Peltier electronics) and magnetic stirring. The antioxidant capacity was expressed as Trolox equivalent antioxidant capacity (TEAC).

Extraction of Phenols from VOO. Extraction of phenols from VOO before and after frying was performed according to the method of Cortesi et al. (*29*). Briefly, olive oil (50 g) was dissolved in hexane (50 mL), and polar compounds were extracted with methanol/water (3:2, v/v, 3 × 30 mL). The final extract was washed with hexane (50 mL), and then the solvent was evaporated under reduced pressure at 40 °C.

The residue obtained was weighed, dissolved in methanol, filtered through 0.2 μm filters (Amicon), and used for determination of total phenols, HPLC separation, for LC-MS analysis.

Determination of Total Phenols Content. The amount of phenolic compounds was given as gallic acid equivalents and determined according to the Folin−Ciocalteu method (*30*). Briefly, 2.5 mL of Folin−Ciocalteu reagent, diluted 10-fold in water, was added to the different VOO phenol extracts. The mixture was incubated for 2 min at room temperature, and 2 mL of sodium carbonate was added. The mixture was incubated for 15 min at 50 °C and finally cooled in a water−ice bath. The specific absorbance at 760 nm was immediately measured.

HPLC-DAD Monitoring of Phenol Composition during Frying. The phenol composition was studied during 4 h of continuous frying carried out using the VOO sample from the Ravece olive variety. HPLC-DAD analysis was carried out on a Shimadzu liquid chromatograph (model LC-10AD) equipped with a diode array detector (model SPD M10A VP). The chromatographic separation was achieved on a 5 μm ODS-3 Prodigy (250 mm × 4.6 mm i.d.) reversed-phase column (Phenomenex, Macclesfield, U.K.) according to the procedure and conditions previously reported (*31*). Solvent A was water/trifluoroacetic acid (97:3). Solvent B was acetonitrile/methanol (80:20). A step gradient from 5 to 98% B (45 min) was applied at a flow rate of 1 mL min^{-1}. Peak quantification was carried out at 279 nm. Major phenol compounds were tentatively identified by comparison with relative retention time of pure compounds and by comparison of the relative elution order and UV spectra with those previously reported (*32*).

LC-MS Analysis of VOO Phenol Extracts. VOO phenolic compounds were tentatively identified by LC-MS, as previously described (*27*). HPLC runs were performed on a Shimadzu instrument configured with two LC-10AD pumps and an SLC10 A control system. Phenolic compound separation was performed on a Biosil C_{18} HL 90-5S column (5 μm, 4.6 × 150 mm) (Bio-Rad). MS analysis was carried out on an API-100 single-quadrupole mass spectrometer (Sciex Instruments) equipped with an atmospheric pressure chemical ionization (APCI) ion source.

The instrument used nitrogen as curtain gas and air to dry the ion spray. Preliminary experiments were also performed using an electrospray ion source (ESI). The HPLC equipment was connected to the MS detector, and chromatographic separation was carried out as described above using a flow rate of 0.8 mL/min. Ionization was achieved at a temperature of 70 °C using a probe voltage of 4.8 kV and a declustering potential of 70 V. The mass-to-charge ratio scale was calibrated with the ions of ammonium adduct of polypropylene glycol. Full-scan spectra were acquired from 100 to 800 atomic mass units (amu) using a step size of 0.5 amu and a dwell time of 2 ms. Single ion monitoring (SIM) was performed simultaneously for four ions: 305, 321, 363, and 379 amu corresponding to the molecular ions (MH^+) of the main phenolic compounds. In these experiments a 500 ms dwell time was selected. Quantitative determination of secoiridoid compounds was obtained using a calibration curve constructed with oleuropein as standard; pinoresinol was quantified using a calibration curve obtained with pure standard.

Frying Experiments. Potatoes (var. Agria) stored at 8 °C and 95% relative humidity were washed and peeled. The slices were cut from the pith of the parenchymatous region of potato tubers using an electric slicing machine (model CL50B). A circular cutting was used to provide chips with a diameter of 30 mm and a thickness of 3 mm. Slices were soaked in water for 1 h and then dried with filter pape; 10 slices (about

30 g) were fried in an electric deep fryer with a capacity of 1 L of oil at the following temperature−time conditions: (i) 180 ± 1 °C for 5 min, (ii) 180 ± 1 °C for 10 min, and (iii) 180 ± 1 °C for 15 min. Oil temperature was monitored by a digital thermometer. After frying, potatoes were cooled, used for color analysis, then homogenized with a mixer, and frozen at −18 °C until used for acrylamide analysis.

Measurement of Color. Color measurements (CIE *L*a*b** color space) were performed using a Konica Minolta CM-3500d reflectance spectrophotometer (Konika Minolta Sensing Inc.). Results report average from the reflectance at both front and rear sides of freshly fried potato readings.

Determination of Acrylamide. Acrylamide was analyzed by LC-MS after methanol extraction and Carrez clarification as described by Gokmen and Senyuva (*33*). Sample (1.00 g) was weighed with a precision of 0.1 mg and suspended with 5 mL of methanol in polypropylene centrifugal tubes. The mixture was spiked with 100 μL of a 5 μg mL^{-1} [$^{13}C_3$]acrylamide methanol solution as internal standard and later homogenized. Acrylamide extraction was performed at room temperature for 20 min. Afterward, the sample was centrifuged (9000*g* for 10 min) at 4 °C, and the supernatant was treated with 25 μL of Carrez I and 25 μL of Carrez II solutions. Supernatant (1 mL) was applied on a rotary evaporator (<40 °C) until dryness and redissolved with water. For sample cleanup, Oasis-HLB (1 mL, 30 mg, Waters, Milford, MA) cartridges were used. Cartridges were preconditioned with 1 mL of methanol, 1 mL of water, and 1 mL of air to remove excess water. An aliquot of the clear supernatant (1 mL) was loaded onto the cartridge at a flow rate of 2 mL min^{-1}. Solution was filtered through a 0.45 μm filter directly into the vial for LC-MS analysis.

Sample extracts and calibration standards were analyzed on an Agilent 1100 liquid chromatograph coupled to an Agilent Quadrupole MS detector (Agilent Technologies, Palo Alto, CA). Analytical separation was achieved with an Inertsil column (25 × 0.46 cm, 5 μm) at 32 °C. Isocratic elution was achieved with a mobile phase of 0.1% acetic acid in water at a flow rate of 0.6 mL/min. Electrospray ionization in positive mode was used. The MS detector operated in selected ion monitoring (SIM) mode at *m/z* ratios of 72.1 and 75.1 for acrylamide and [$^{13}C_3$]acrylamide respectively. Under these chromatographic conditions, acrylamide eluted at 11.7 min. The needle and cone voltages were set at 3.0 kV and 100 V, respectively. Nitrogen was used as nebulizer gas (12.0 L h^{-1}), and the source temperature was set at 300 °C. Acrylamide was quantified using a calibration curve obtained with a standard solution of acrylamide and [$^{13}C_3$]acrylamide. Acrylamide contents in sample extracts were calculated by taking into account the recovery calculated by means of [$^{13}C_3$]acrylamide slope. The limit of quantitation was set at 30 μg kg^{-1}.

Statistical Analysis. All data were subjected to analysis of variance (ANOVA). The general linear model SPSS statistical package was used for the evaluation of statistical significance of the differences between mean values by Tukey's test and for the calculation of Pearson correlation coefficients between different variables.

RESULTS AND DISCUSSION

Characterization of VOO Phenolic Compounds. The phenolic compound profile of VOO is complex and dependent on olive variety, climate, and maturation degree (*34*). Data of phenolic compounds regarding the 20 VOO samples screened in this study are shown in **Table 1**. The median total phenols content was 344 ppm (between 148 and 642 ppm), which is slightly above the range of the usual value reported for VOO (between 100 and 300 ppm) (*35, 36*). The TEAC value ranged between 2.24 ± 0.19 and 7.28 ± 0.12 (Ravece oil). As expected, there is a positive correlation between TEAC and total amount of phenolic compounds. These values of antioxidant activity are slightly higher than those previously reported for VOO activity (*28*), but they are typical of freshly made VOO, in which the phenolic compounds are not hydrolyzed as usually happens during storage.

The phenol profile of the various VOO samples was analyzed by HPLC-MS, and the chromatogram of a representative VOO sample is shown in **Figure 1**. The MS tentative identification of the main peaks of the chromatogram was highlighted. Particularly, the main phenol compounds considered were the oleuropein aglycon (3,4 DHPEA-EA), the ligstroside aglycon (p-HPEA-EA), the dialdehydic form of elenolic acid linked to hydroxytyrosol (3,4-DHPEA-EDA), and the dialdehydic form of elenolic acid linked to tyrosol, (p-HPEA-EDA). The last compound is of particular interest as it is mainly responsible for the throat-irritating sensation (*36*); it was recently renamed oleocanthal and, considering its structural similarity with ibuprofen, indicated as being responsible for the anti-inflammatory properties of the Mediterranean diet (*37*). Other interesting phenolic compounds in VOO are two lignans, namely, pinoresinol and deacetoxypinoresinol (*38*): the former was present in relevant amount, and it was also quantified in the samples used in this study.

Table 1. Phenol Content and Antioxidant Activity of the Virgin Olive Oil (VOO) Samples Used in This Study[a]

sample	total phenolic content (gallic acid equiv, mg kg^{-1})	TEAC
1	148 ± 3	3.91 ± 0.22
2	152 ± 1	2.55 ± 0.10
3	169 ± 1	2.98 ± 0.03
4	438 ± 1	4.91 ± 0.49
5	212 ± 4	4.04 ± 0.18
6	250 ± 4	5.05 ± 0.19
7 (Iblea 7)	**602 ± 3**	**5.29 ± 0.49**
8	312 ± 5	2.24 ± 0.19
9	446 ± 1	2.64 ± 0.15
10	188 ± 3	2.89 ± 0.18
11 (Iblea 11)	**592 ± 3**	**3.59 ± 0.18**
12	446 ± 3	4.56 ± 0.29
13	590 ± 2	3.65 ± 0.18
14 (Ravece)	**642 ± 3**	**7.28 ± 0.12**
15	428 ± 1	2.40 ± 0.10
16	260 ± 1	2.85 ± 0.07
17	366 ± 1	2.71 ± 0.26
18 (Caiazzana)	**284 ± 3**	**3.04 ± 0.12**
19	344 ± 4	3.13 ± 0.28
20	220 ± 3	3.13 ± 0.10

[a] Phenol content was expressed as gallic acid equiv, mg kg^{-1}. Antioxidant activity of VOO was expressed as TEAC (Trolox equivalent antioxidant capacity), mmol of Trolox kg^{-1} of product. The samples selected for the frying experiment are given in bold.

Among the samples of **Table 1** four oils, having different phenolic patterns, were selected for frying experiments. In **Table 2** the concentration of the main phenolic compounds of these four VOO samples is reported. The two oils derived from Iblea olives, a variety typical of eastern Sicily, have similar concentrations of all secoiridoid derivatives, but Iblea 11 has a lower pinoresinol concentration than Iblea 7. The other two VOO samples were obtained from two olive varieties typical of the Campania region: Ravece oil has similar total phenol compounds with respect to the Iblea oils but the highest concentration of oleuropein derivatives (namely, the two dihydroxyphenol compounds). As a consequence, its antioxidant activity is very high, although the total amount of phenolic compounds is not significantly higher than those of the two Iblea samples. In contrast, an oil obtained from Caiazzana olives was selected. This is a very sweet oil, poor in all secoiridoid derivatives. On the other hand, it has a concentration of pinoresinol similar to that of the other oil samples.

In summary, among the four oils selected three oils have similar high amounts of secoiridoid compounds but different

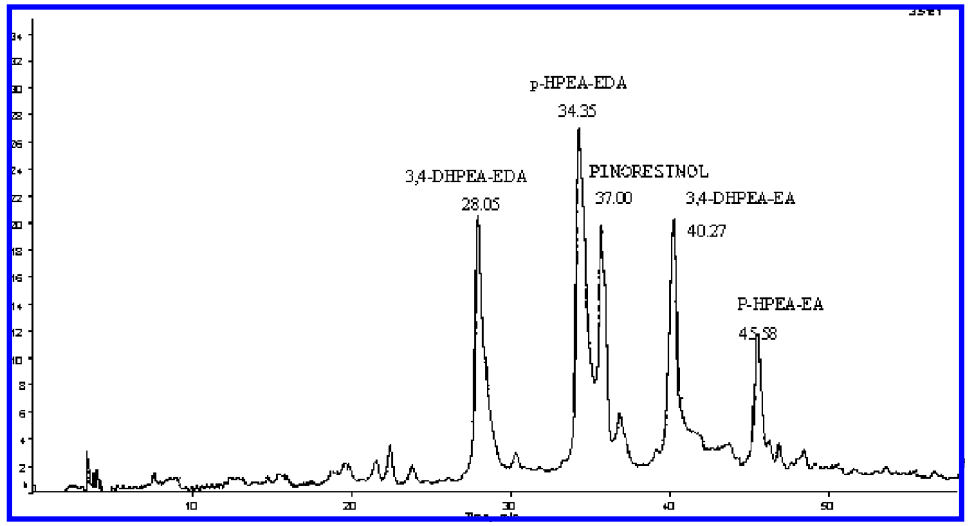

Figure 1. TIC chromatogram of the phenolic compounds extracted from Ravece virgin olive oil (VOO).

Table 2. Amount (mg kg^{-1}) of the Main Phenol Compounds Present in the Four Virgin Olive Oils (VOO) Used for Frying Experiments[a]

VOO sample	p-HPEA-EDA	p-HPEA-EA	3,4-DHPEA-EDA	3,4-DHPEA-EA	pinoresinol	dihydroxy/monohydroxy
Iblea 7	62.7 ± 0.9	33.1 ± 1.0	10.6 ± 1.2	63.4 ± 1.09	23.0 ± 0.7	0.8
Ravece	54.7 ± 1.0	10.8 ± 1.2	75.5 ± 1.2	55.5 ± 0.5	29.1 ± 0.5	2.0
Caiazzana	2.5 ± 1.2	7.0 ± 0.8	1.1 ± 1.09	7.4 ± 1.09	29.4 ± 0.6	0.9
Iblea 11	70.1 ± 0.5	34.5 ± 1.1	37.5 ± 0.7	53.5 ± 0.8	11.7 ± 0.8	0.9

[a] In the last column is reported the ratio between dihydroxy and monohydroxy phenol compounds. Abbreviations: p-HPEA-EDA, dialdehydic form of elenolic acid linked to tyrosol; p-HPEA-EA ligstroside aglycon; 3,4-DHPEA-EDA, dialdehydic form of elenolic acid linked to hydroxytyrosol; 3,4 DHPEA-EA, oleuropein aglycon.

ratios between dihydroxy and monohydroxy derivatives; ;this ratio is below 1 in the Iblea oils and about 2 in the Ravece oil. The fourth oil is poor in secoiridoids but has the same amount of pinoresinol.

Frying Experiments: Oil Stability and Color Development. The four oils above-described have been used to verify our working hypothesis about the possible relationship between antioxidant phenolic compounds present in the frying oil and acrylamide formation in crisps. Three different frying times were selected to cover mild (5 min), moderate (10 min), and severe (15 min) potato deep-frying conditions. In these conditions, as shown in **Figure 2**, the loss of phenols in the first 15 min of the frying process is negligible. To observe a significant loss of secoiridoid derivatives a much longer frying time should be used: after 4 h of frying, 3,4-DHPEA-EDA decreased by about 50%, whereas 3,4-DHPEA-EA showed a decrease of about 70%. A similar behavior was also reported in other studies (*39*). This confirms previous data about VOO phenolic compounds' thermal stability (*40*) and previous frying tests performed in our laboratory showing a good thermal stability of VOO phenol compounds.

Color is an important quality criterion for the potato-processing industry, which is the result of Maillard reaction at the surface, and it is strictly related to consumer perception (*41*). In **Figure 3** the color values of potatoes fried at 180 °C for three different times, 5, 10, and 15 min, are shown. In all cases, browning increased with time. The *L** values, luminance or lightness component, at 10 min of frying are slightly higher in the Iblea 11 sample, but for all other times no significant differences among the samples were detectable. The trend of *L** decreasing with time was in accordance with other authors (*42*). These results suggest that the presence of different concentrations of phenolic compounds in the oils had no direct effect on the browning process during the frying of potato samples and, subsequently, on consumer perception.

The sensory profile of crisps fried in VOO was not investigated in this study. Sensory tests previously carried out on French fries using VOO as frying medium have indicated the acceptability of fried potatoes it is not affected by the use of VOO, which ensures good texture and flavor of fried foods (*43*). The fat uptake of foods fried in VOO is comparable with that deriving from the use of other vegetable frying oils (*44, 45*).

However, the use of phenol-rich VOOs influences the overall flavor and taste of fried crisps. The major problem in sensory acceptability of fried crisps could be the possible contribution of bitter phenols (aglycons of secoiridoids) to the taste of the crisps. However, the behavior of bitter aglycons of secoiridoids during thermal treatments in the presence of water has been already studied in model systems and during the deep-frying of French fries (*31, 39*). These studies showed that simple phenyl alcohols (tyrosol and hydroxytyrosol) are preferentially absorbed by potatoes, whereas the absorption of bitter aglycons (3,4-DHPEA-EDA, 3,4-DHPEA-EA, HPEA-EDA, and HPEA-EA) is negligible. In fact, at high temperatures and in the presence of water (the microenvironment of the crust interface during deep frying) bitter aglycons are rapidly hydrolyzed in nonbitter simple phenyl alcohols and elenolic acid (*31, 39*). Therefore, even after frying with bitter VOO rich in phenols, fried potatoes do not have strong bitter attributes and their acceptability is not negatively affected.

Frying Experiments: Acrylamide Formation. It has been recently demonstrated that frying oils used in deep-frying practices are not an external source to contamination of foodstuffs with acrylamide, so potential formation of acrylamide from olive oil was ruled out (*46*).

The data of acrylamide concentration in potato crisps are summarized in **Figure 4**. Acrylamide formation rapidly increased with frying time as repeatedly demonstrated, and its concentration is in line with those reported in the literature for potato crisps at similar time–temperature combinations (*15, 38, 47*). However,

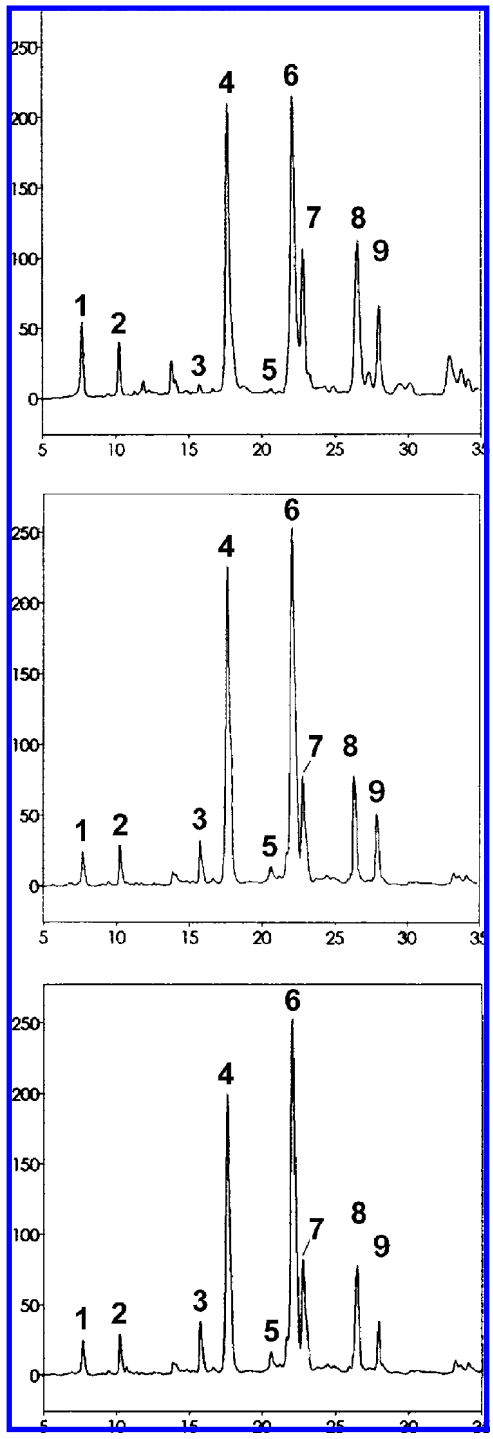

Figure 2. HPLC-UV chromatograms (279 nm) of the phenolic compounds obtained from unheated Ravece VOO: top, unheated; middle, after 1 h of frying at 180 °C; bottom, after 4 h of frying at 180 °C. Peaks: 1, hydroxytyrosol (3,4-DHPEA); 2, tyrosol (p-HPEA); 3, unknown; 4, 3,4-DHPEA-EDA; 5, unknown; 6, p-HPEA-EDA; 7, pinoresinol; 8, 3,4-DHPEA-EA; 9, p-HPEA-EA.

when the crisps fried in different oils at the same time were compared, significant differences were found. Crisps fried in Caiazzana oil have the highest rate of acrylamide formation. After 5 min of frying, the amount of acrylamide was relatively low (between 350 and 720 μg kg^{-1}) in potatoes fried with Ravece and Iblea oils, but quite high in the Caiazzana, reaching a value around 1800 μg kg^{-1}. As Caiazzana is the oil having the lowest concentration of phenolic compounds and particularly of secoiridoid derivatives, this is an indication that these

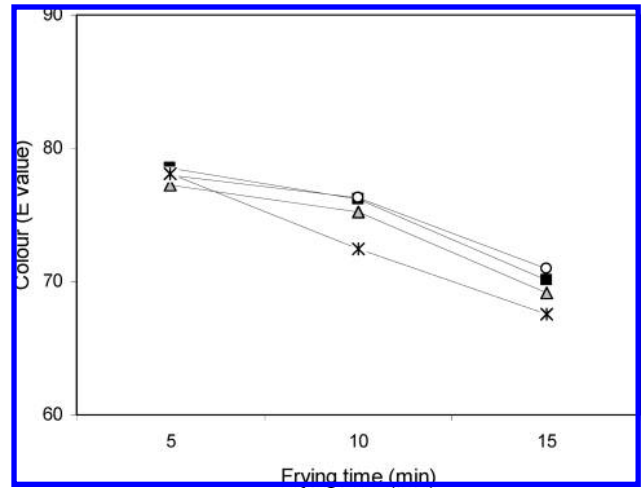

Figure 3. Change of potato crisps CIE L^* during frying at 180 °C for three different times (5, 10, and 15 min) in the four different oils: (solid square) Iblea 11; (open circle) Caiazzana; (open triangle) Ravece; (asterisk) Iblea 7.

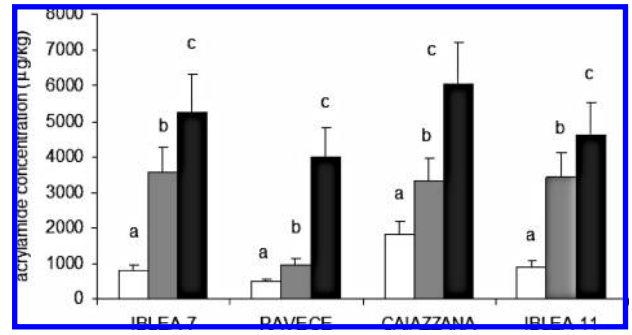

Figure 4. Acrylamide concentration of potato crisps fried at 180 °C for different times in the four VOO samples: (white bars) 5 min; (gray bars) 10 min; (black bars) 15 min. For each oil, different letters indicate significantly different values.

compounds play a role in preventing acrylamide formation in potatoes during frying.

From a comparison of the other three oil samples it is clear, particularly at 10 min of frying, that Ravece oil is the best performer in delaying acrylamide formation. In fact, crisps fried for 10 min in Ravece oil have an acrylamide concentration of 800 μg kg^{-1}, whereas all others exceeded 3000 μg kg^{-1}. Looking at the phenolic compound patterns of these three oils as reported in **Table 2**, this effect should be attributed to the two dihydroxyphenol compounds (3,4 DHPEA-EA and 3,4-DHPEA-EDA), which are present in higher relative amount in Ravece oil than in the two Iblea oils. Data of **Figure 5** showed a significant inverse correlation between the concentration of acrylamide in crisps after 5 min and the amount of 3,4-DHPEA-EDA plus 3,4 DHPEA-EA. The correlation is not significant when acrylamide data at 10 and 15 min of frying were considered.

A different hypothesis can be formulated to explain the finding that olive oil having a high concentration of polar antioxidants is able to reduce acrylamide formation during potato frying. A similar ability of VOO phenolic compounds has been observed in the reduction of heterocyclic amine (HA) formation in fried meat burgers (*26*). In that system the observed effect was attributed to the ability of VOO antioxidants to block the radical-involving pathway leading to HA formation. In the case of acrylamide formation in potato it is known that there is not a radical reaction involved; however, we should consider that

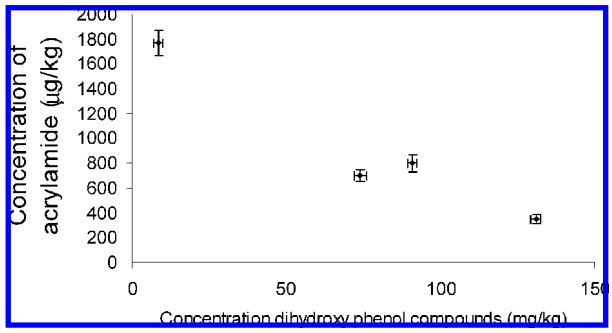

Figure 5. Correlation between the amount of acrylamide formed in potato crisps after 5 min of frying and the amount of dihydroxyphenolic compounds (3,4-DHPEA-EDA plus 3,4-DHPEA-EA) in the oil.

the overall MR is a carbohydrate oxidation reaction that in some cases is inhibited by antioxidants (*48*). Moreover, during the conversion of asparagine into acrylamide there is a decarboxylation step producing CO_2, which is again an oxidation reaction. The ability of polar antioxidants to inhibit oxidation in lipophilic media is well described (*25, 49*) and attributed to the ability of these compounds to act at the interface between the oil and the polar phase, and this is exactly the region where the acrylamide is formed during potato frying. In other words, frying with oil having a high concentration of polar compounds could reduce acrylamide formation indirectly by maintaining a reducing environment, which in turn inhibits acrylamide formation.

Previous studies found controversial results on the effect of the type of frying oil on acrylamide formation during deep-frying of potato crisps. Vegetable oils from different sources have been studied, but their antioxidant properties or the concentration and chemical characterization of the reducing compounds has not been considered (*21, 50*). The findings of this paper indicate that the oil used for frying should not be considered only as an heat transfer medium: using VOOs with defined phenol compound compositions, significant differences in the acrylamide formation can be observed. In particular, acrylamide formation was delayed when a VOO rich in dihydroxyphenolic compounds was used.

The use of phenol-rich VOO can be proposed as a good mitigation strategy to reduce acrylamide formation in fried potatoes. Frying using virgin olive oils cannot be proposed for industrial use, but it is a common practice in domestic deep-frying in all Mediterranean countries, which could be exported to other areas of the world.

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