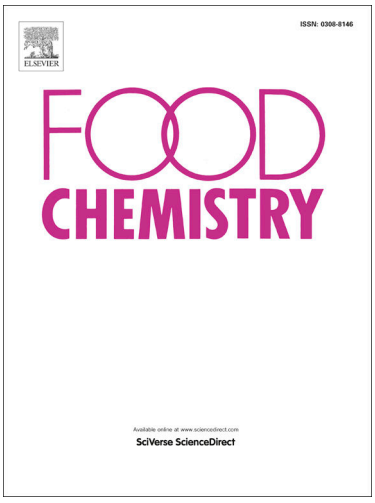

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Spray-dried olive mill wastewater reduces Maillard reaction in cookies model system

*Antonio Dario Troise [1,2], Antonio Colantuono [1,3] and Alberto Fiore [1,4]**

[1] Department of Agricultural Sciences, University of Naples, "Federico II", 80055, Portici, Italy

[2] Proteomics & Mass Spectrometry Laboratory, ISPAAM, National Research Council, Naples, Italy

[3] Arterra Bioscience S.p.A., 80142, Napoli, Italy

[4] Division of Engineering and Food Science, School of Applied Science, Abertay University, Dundee DD1 1HG, UK

***Corresponding author**

Alberto Fiore, a.fiore@abertay.ac.uk

Abstract

The network of the Maillard reaction can be influenced by the presence of polyphenols. In this paper, we evaluated the ability of secoiridoids to interact with asparagine and lysine tuning the formation of dietary advanced glycation end-products (d-AGEs), dicarbonyls and acrylamide. Olive oil mill wastewater polyphenol powders (OMWP) were added to glucose and lysine or asparagine in silica model systems to mimic water activity present in cookies. Results revealed that acrylamide, Amadori compounds and *N*-ε-carboxyethyllysine (CEL) were reduced to 50%, after 13 min at 180°C; for the reduction of *N*-ε-carboxymethyllysine (CML), secoiridoids were effective only in model systems with the addition of acacia fiber and maltodextrin as coating agents. In cookies, OMWP at three different concentrations decreased the concentration of protein bound Amadori compounds, CML, CEL and dicarbonyls. Acrylamide and 5-hydroxymethylfurfural were reduced to 60% and 76% respectively, highlighting the ability of secoiridoids-based functional ingredients in controlling d-AGEs formation.

Keywords: Maillard reaction, acrylamide, d-AGEs, polyphenols**,** olive mill wastewater

List of chemical compounds:

N-ε-(2-furoylmethyl)-L-lysine (furosine, PubChem CID: 123889); *N*-ε-carboxymethyllysine (CML, PubChem CID: 123800); *N*-ε-carboxyethyllysine (CEL, PubChem CID: 23400779); Lysine (PubChem CID: 5962); Asparagine (PubChem CID: 6267); Acrylamide (PubChem CID: 6579); Acrylamide-d3(PubChem CID: 12209671); 5-Hydroxymethylfurfural (HMF, PubChem CID: 237332); *N*-(1-deoxy-D-fructos-1-yl)-L-lysine (PubChem CID: 123708); 2-(4-hydroxyphenyl)ethanol (tyrosol, PubChem CID: 10393); 3-hydroxytyrosol (Hydroxytyrosol, PubChem CID: 82755); Verbascoside (PubChem CID: 5281800); Glyoxal (PubChem CID: 7860); Methylglyoxal (PubChem CID: 880); 2,3-butanedione (diacetyl, PubChem CID: 650).

1. Introduction

The Maillard reaction (MR) includes a series of complex and interrelated chemical reactions that have significant implications for color, flavor and texture of many thermally treated foods (Hellwig & Henle, 2014). In the early stage of MR, amino acids, peptides and proteins react with carbonyl groups of reducing sugars, resulting in Schiff base formation and rearrangement to Amadori products (APs) or Heyns products (HPs). The degradation of APs and HPs leads to the formation of furfural and 5-hydroxymethylfurfural (HMF) or alternatively, depending on the pH, to the formation of α-hydroxycarbonyls and α-dicarbonyls. APs and HPs are involved in flavor generation via Strecker degradation (van Boekel, 1998), although α-dicarbonyl compounds may react with the ε-amino group of lysine or arginine to form dietary advanced glycation products (d-AGEs), including *N*-ε-carboxymethyllysine (CML), *N*-ε-carboxyethyllysine (CEL) and other hydroimidazolone derivatives.

While the intake of d-AGEs has controversial physiological consequences on the influence of protein digestibility (Delgado-Andrade & Fogliano, 2018), food-derived α-dicarbonyls are recognized to enhance oxidative stress and their restriction in the diet has been proposed as a strategy for alleviating complications of diabetes and metabolic syndromes (Hellwig, Gensberger-Reigl, Henle & Pischetsrieder, 2018). Along with α-dicarbonyls, acrylamide formation in foods represents a potential health concern. In the first full risk assessment of acrylamide in foods, the European Food Safety Agency (EFSA) confirmed the findings of previous evaluations on the relationship between acrylamide in foods and the increased cancer risk for consumers in all age groups, ('EFSA', 2015). In this context, several technological approaches have been proposed that aim to reduce the amount of acrylamide, α-dicarbonyls and d-AGEs in thermally treated foods. The most effective include the use of alternative cooking treatments and processing methods to reduce the thermal loading, as well as control of storage conditions, enzymatic strategies and the addition

of new ingredients such as encapsulated reactants and polyphenols-based additives (Lund & Ray, 2017).

Polyphenols from olive by-products can be included as functional molecules in food products and have gained increasing interest as natural molecules are usually more accepted as food ingredients than synthetically manufactured compounds (Nunes, Pimentel, Costa, Alves & Oliveira, 2016). Beyond their beneficial effects on human health, several previous studies have demonstrated that polyphenols are able to interact with Maillard precursors, intermediates and end-products both in model systems and in foods. Specifically, it has been hypothesized that aromatic ring or nucleophiles can exert their effects through three different mechanisms: 1) by trapping α-dicarbonyls via polyphenols aromatic rings; 2) by blocking the synthesis of pyrazinium radicals influencing the formation of eneaminol radical precursor; 3) by breaking the cross-linking structures in the formed d-AGEs and by inhibiting the formation of Amadori products through the reaction of quinone rings with free amino groups (Bin, Peterson & Elias, 2012, Totlani & Peterson, 2006, Yeh, Hsia, Lee & Wu, 2017).

Olive mill wastewater (OMW) is a major by-product of the olive oil production process and represents an abundant source of polyphenols, among which the most relevant are secoiridoid derivatives, in particular hydroxytyrosol, the dialdehydic form of decarboxymethyl oleuropein aglycone, tyrosol and verbascoside (De Marco, Savarese, Paduano & Sacchi, 2007). Recently, OMW has been proposed for the formulation of functional foods and ingredients with a wide range of effects. Specifically, polyphenols from OMW act as natural food antioxidants, oxidative stabilizers, texture enhancers in food emulsions as well as antimicrobial molecules in meat products (Araújo, Pimentel, Alves & Oliveira, 2015). Furthermore, previous studies demonstrated the ability of a polyphenols-rich extract from OMW in preserving α-tocopherol content and reducing the production of negative volatile compounds, in a refined olive oil during a frying process (Esposto *et al*., 2015). The carbonyl trapping and antiglycative capacities of phenols from OMW were also

investigated in foods, pointing to a potential role of OMW-derived ingredients in the reduction of reactive carbonyl species, MR end-products and MR derived off-flavors in ultrahigh-temperature-treated (UHT) milk (Troise, Fiore, Colantuono, Kokkinidou, Peterson & Fogliano, 2014). Regarding molecular level, hydroxytyrosol can effectively trap glyoxal and methylglyoxal, which together with 3-deoxyglucosone, represents the most abundant dicarbonyls in foods (Navarro, Atzenbeck, Pischetsrieder & Morales, 2016). Moreover, among the different tuning strategies, the interplay between MR and lipid oxidation can be considered as one of the most relevant targets for the addition of polyphenols (Zamora & Hidalgo, 2016).

Framed by the relationship between acrylamide, d-AGEs and polyphenols, in this work we evaluated the ability of spray-dried ultra-filtrated OMW in controlling free lysine and asparagine modifications and HMF formation in silica model systems and the time evolution profile was used as a reference to monitor d-AGEs, HMF, acrylamide and dicarbonyls formation in a cookies model system.

2. Material and methods

2.1. Chemicals and reagents

Acetonitrile, methanol, and water were obtained from Merck (Darmstadt, Germany). The ion pairing agent perfluoropentanoic acid, trichloroacetic acid, hydrochloric acid (37%), the analytical standards L-lysine hydrochloride, [4,4,5,5-*d4*]-L-lysine hydrochloride (*d4*-Lys), tyrosol (2-(4-hydroxyphenyl)ethanol, 98%), 3-hydroxytyrosol 98%, and verbascoside 98% along with 5-hydroxymethylfurfural , glyoxal 40% solution in water, methylglyoxal 40% solution in water, and 2,3-butanedione (diacetyl, 97%) as well as *o*-phenylenediamine (OPD, 99.5%), diethylenetriaminepentaacetic acid (DETAPAC), quinoxaline (QX), 2-methylquinoxaline (2-MQX), 2,3-dimethylquinoxaline (2,3-MQX), asparagine and formic acid (MS grade 98%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Analytical standards *N*-ε-(2-furoylmethyl)-

L-lysine (furosine) and *N*-ε-(2-furoyl[$^2H_4$]methyl)-L-lysine (*d4*-furosine) were obtained from Polypeptide laboratories (Strasbourg, France). *N*-ε-carboxymethyllysine (CML), *N*-ε-(carboxy[$^2H_4$]methyl)-L-lysine (*d4*-CML), *N*-ε-carboxyethyllysine (CEL), *N*-ε-(carboxy[$^2H_4$]ethyl)-L-lysine (*d4*-CEL), *N*-(1-deoxy-D-fructos-1-yl)-L-lysine (FL) and [2,3,3-*d3*]-acrylamide were obtained from TRC (Toronto Research Chemicals, Ontario, Canada).

2.2. Olive oil mill wastewater polyphenol powders (OMWP) preparation

Olive oil mill wastewater polyphenol powders (OMWP) were prepared according to Navarro *et al*. (2015) and Troise *et al*. (2014). In summary, OMW was obtained from virgin-olive oil production (Carolea variety). After the centrifugation step to remove the oil and the oil paste, the aqueous fraction was incubated with pectinase for 2 hours at 37°C and followed by different filtration steps including microfiltration (cutoff 25 kDa), ultrafiltration (cutoff 8 kDa), nanofiltration (cutoff 0.3 kDa), and reverse osmosis (cutoff 0.1 kDa). Retentate of ultrafiltration was collected and spray dried adding maltodextrin and acacia fiber in a ratio 2:1 of the water mill dry weight (66.6% olive mill water and 33.3% maltodextrin and acacia fiber 1:1). The spray drier (CIBEC SpA Industrie, Maranello, Italy) was used at 165 °C for the inlet temperature and <80 °C for the outlet temperature, the feeding was set at 4.5 L/h, the turbine was set at 2700 x *g*. The moisture of the final product was 7%.

2.3. OMWP characterization

The analysis of the phenolic component in OMWP was carried out as previously described with some modifications (Navarro, Fiore, Fogliano & Morales, 2015). Briefly, the OMWP was dissolved in distilled water, in order to prepare a solution at a concentration of 20 mg/mL, spiked with 10 µL of a 5 mg/L solution of butyl-4-hydroxybenzoate as the internal standard. The phenolic fraction was purified through polymeric Strata X C18 cartridges (Phenomenex, Torrance, CA), previously activated with methanol and water. Cartridges were further washed with 3 mL of water, and 1 mL of methanol was collected and dried under a gentle nitrogen stream. The purified fraction was

dissolved in 500 µL of a solution of 5% methanol in water. An LC-20AD HPLC with a UV-Vis detector SPD20A, set at 279 nm, and a SIL-20A autosampler (Shimadzu, Kyoto, Japan) was used, while chromatographic separation was achieved through a Prodigy ODS3 column (250 × 4.60 mm, 5 µm, Phenomenex). Mobile phases consisted of water (A) and methanol (B) and the following gradient was used: 0 min (5% B); 4 min (5% B); 40 min (98% B); 43 min (98% B). Hydroxytyrosol, tyrosol and verbascoside were quantified by external calibration technique according to the procedure detailed by Navarro and coworkers (Navarro, Fiore, Fogliano & Morales, 2015).

2.4. Silica model systems

Binary mixtures of glucose (18 mg) and either asparagine (13.2 mg) or lysine (14.6 mg) were homogenized with silica gel (5g; KG 60, 0.063-0.200 mm) containing 4% of water (214 mg), 37 mg of sodium chloride, 15 mg of ammonium hydrogen carbonate, 30 mg of sodium hydrogen carbonate. Two other samples were prepared by adding to the above-mentioned system 6 mg of OMWP (0.1%) and 6 mg of a mixture of acacia fiber and maltodextrin 50:50, w/w (0.1%) respectively. Then the samples were heated at 180 °C in closed glass vessels for 5, 7, 9, 11 and 13 min. After cooling at room temperature, 5 mL of water was added and the mixture was stirred for 15 min. After an ultrasound-assisted bath (4 min), the suspension was centrifuged (2700 x *g*; 10 min at 10 °C) and the supernatant was filtered (RC filters, Phenomenex) for direct analysis of precursors (asparagine and lysine), intermediates (FL) and end-products (acrylamide, CML and CEL) as described below.

2.5. Cookies model systems

Cookies were prepared according to the method described by Fiore *et al*. (2012) with some modification. In summary, three different concentrations of OMWP (0.05%, 0.1% and 0.2%) were added to cookie doughs and mixed with the others ingredients. Control cookies were prepared without OMWP and a control sample plus coating agents were prepared by adding a mixture of 160

mg of acacia fiber and maltodextrin 50:50, w/w (**Supplementary table 1**) to the formulation. Each ball of dough was rolled between two bars with a height of 3 mm and shaped in a disk of 30 mm diameter to achieve maximum homogeneity among the various batches. Subsequently, the cookies were baked at 180°C for 13 min in a forced-air circulation oven (Memmert, Schwabach, Germany)

2.6. Acrylamide analysis

Acrylamide was analyzed according to Troise *et al.* with slight modifications (Troise, Fiore & Fogliano, 2014). Freeze dried cookies were pulverized in a knife mill Grindomix 200 (Retsch, Haan, Germany) and 0.5 g of powder was weighed; then 3 mL of deionized water was added along with 100 µL of Carrez reagent potassium salt, 100 µL of Carrez reagent zinc salt and 10 µL of internal standard [2,3,3-*d3*]-acrylamide (final concentration 90 ng/mL). The tubes were shaken vigorously for 5 min at 400 rpm. The resulting mixture was centrifuged at 2700 x *g* for 10 min at 4 °C. The supernatant was then collected in a 15 mL volumetric flask and two extraction cycles were performed using 3 mL of deionized water for each cycle. Finally, the pellets were discarded, and the supernatants were filtered through a 0.45 µm modified cellulose filter (Phenomenex, Torrance, CA). An aliquot of 1 mL was passed through an Oasis HLB cartridge (Waters, Milford, MA) previously activated with 1 mL of methanol and 1 mL of deionized water; 10 µL of the final solution was injected onto the chromatographic column for quantitation by tandem mass spectrometry (MS/MS). Identification and quantification of acrylamide and *d3*-acrylamide were carried out using an API 2000 triple-quadrupole mass spectrometer (Applied Biosystems, Carlsbad, CA) coupled to an ion spray interface, equipped with an HPLC binary micropump series 200 (Perkin-Elmer, Waltham MA). Chromatographic separation of acrylamide and *d3*-acrylamide was achieved through a Synergi Hydro (150 x 2.0 mm, 4.0 µm, Phenomenex) equipped with a C18 security guard; the mobile phases were as follows: A, 0.1% formic acid and B, 0.1% formic acid in methanol. The gradient elution (min)/(% B) used was: (0/5), (3.5/5), (7/55), (8/55) at a flow rate of 0.2 mL/min. Acrylamide and its labeled internal standard were analyzed using the mass transitions given in

parentheses and in bold the transition used for the quantitation (target analytes) and qualification (internal standard): acrylamide (*m/z* 72 → **55**, 44, collision energy (CE): 22 and 14), *d3*-acrylamide (*m/z* 75 → 58, **44**, CE: 23 and 18). The quantitation was carried out in multiple reaction monitoring (MRM) with the following conditions: the source temperature was set at 350 °C and the spray voltage was set at 5.0 kV. Under the above-mentioned chromatographic conditions, acrylamide and its labelled standard eluted at 3.4 min. Analytical performances were tested according to (Troise, Fiore & Fogliano, 2014). For the silica model system, asparagine transitions were included along with acrylamide and *d3*-acrylamide transitions. In particular by using the same analytical setup, asparagine quantitation was carried out in MRM by using the following transitions, with the one in bold used for quantitation: 133 →**87** CE: 20 V, 133 → 74, CE: 18 V. Typical retention time was 2.9 min and a calibration curve was performed with the external standard technique in the range of 25-5000 ng/mL.

2.7. HMF analysis

HMF was determined according to Fiore *et al*.(2012) with minor modifications. Following the procedure reported above for acrylamide extraction, clarified supernatants were filtered through a 0.45 µm modified cellulose filter (Phenomenex), and 1.5 mL was collected and used for HPLC analysis. The HPLC system consisted of LC-20AD class VP pumps and an SPD-M20A UV-Vis detector equipped with a SIL-20A autosampler, all from Shimadzu (Kyoto, Japan). Separation of HMF was achieved on a Synergi Fusion-RP 4.0 µm, 100 × 2.0 mm column (Phenomenex), using the following mobile phases: water (A) and acetonitrile (B). HMF was eluted at 400 µL/min through the following gradient of solvent B (t in [min]/[%B]): (0/2), (5/2), (7/40), (12/40). The UV detector was set at 280 nm and HMF was quantified using the external standard technique. The typical retention time was 5.9 min.

2.8. *Lysine modifications analysis*

Lysine and its reducing sugar-mediated modifications, namely furosine, CML and CEL were simultaneously analyzed following procedure described in Troise *et al*. (2018). After HCl (6 M) hydrolysis, the mixture was filtrated by polyvinylidene fluoride filters (PVDF, 0.22 µm Millipore, Billerica, MA) and 200 µL was dried under nitrogen flow. Samples were dissolved in 190 µL of water and 10 µL of the internal standard mix (d_4-lysine, d_4-furosine, d_2-CML and d_4-CEL was added in order to obtain a final internal standard concentration of 200 ng/mg of the samples. Upon loading Oasis HLB 30 mg cartridges (Waters, Wexford, Ireland), target analytes were eluted with 20 mM NFPA: methanol (50/50, v/v) and the collected fraction was dried by using a Savant centrifugal evaporator (Thermo Fisher Scientific, Bremen, Germany). The samples were dissolved in 200 µL of 50% acetonitrile and 5 µL was injected. Separation of furosine, CML, CEL, lysine and their respective internal standards was achieved on a core shell HILIC column (Kinetex HILIC, 2.6 µm, 75 mm x 2.1 mm, Phenomenex) using the following mobile phases: A, 90/10 acetonitrile/50 mM ammonium formate pH 3.5 and B, 50/40/10 acetonitrile /water/50 mM ammonium formate pH 3.5, v/v/v. Both mobile phases were acidified with formic acid, 0.05%. The compounds were eluted at 300 µL/min through the following linear gradient of solvent B (t in [min]/[%B]): (0/0), (2/0), (7/100), (9/100). Positive electrospray ionization was used for detection, and the source parameters were selected as follows: spray voltage, 5.0 kV; capillary temperature, 350 °C; dwell time, 100 ms; cad gas and curtain gas were set to 45 and 5 (arbitrary units), respectively. The chromatographic profile was recorded in MRM by using an API 3000 triple quadrupole (ABSciex). Analytical performances, mass transitions, robustness, sensitivity, reproducibility, repeatability, linearity, accuracy, carry over and matrix effects were evaluated by following procedures reported by (Troise, Wiltafsky, Fogliano & Vitaglione, 2018). For the silica model system, CML, lysine, and CEL were monitored through the external standard technique along with FL upon filtration of the reaction mixture. By using the same chromatographic conditions described above, FL was monitored

through the following transitions (133 →291 CE: 18 V, 309 → **84**, CE: 25 V), with the transition in bold used for quantification.

2.9. Dicarbonyls detection and quantitation

Dicarbonyls were quantified upon derivatization with OPD according to the method described by Degen *et al.*, with slight modifications (Degen, Hellwig & Henle, 2012). Cookie samples (0.5 g) were mixed with water and vortexed at room temperature (10 min, 1000 rpm), then 3 mL of methanol was added. After incubation at -20 °C for 1 h, samples were centrifuged (2700 x *g,* 4 °C) and the hydroalcoholic supernatants were collected for further purification. Polymeric C18 cartridges (Phenomenex) were activated with 1 mL each of methanol and water; 1 mL of clarified supernatants spiked with butyl 4-hydroxybenzoate (500 ng/mL final concentration) was passed through and collected along with 1 mL of water. Samples and aqueous fractions were combined and 0.5 mL was used for derivatization with OPD (0.2% in 9.6 mM DETAPAC). Before incubation at 37 °C for 4 h, samples were combined with 0.15 mL of sodium phosphate buffer (20 mM, pH 7). Determination of three quinoxaline derivatives were performed on a LC-20AD class VP pumps and an SPD-M20A UV-Vis detector equipped with a SIL autosampler (Shimadzu). A Kinetex C18 column (150 mm, 2.1 mm, 2.6 µm; Phenomenex) equipped with a security guard of the same stationary phase was used. Separation was achieved through a gradient mixture of (A) 0.1% acetic acid in water and (B) 0.1% acetic acid in methanol at a flow rate of 0.2 mL/min. The following gradients of solvent B were used (t in [min]/[%B]): (0/5), (5/5), (15/80), (17/80). The UV detector was set at 313 nm and the retention times of QX, 2-MQX and 2,3-MQX were 9.12, 11.13 and 12.03 min, respectively.

2.10. Statistical analysis

All experiments were performed in quadruplicate unless otherwise stated. Each recipe was independently prepared twice and samples from each batch were analyzed twice for a total of four

replicates. Significant differences ($p < 0.05$) of samples were analyzed by Tukey's multiple comparisons test (GraphPad, Prism, La Jolla, CA).

3. Results and discussion

3.1. Silica gel model system

The silica gel model systems were prepared by taking into account the water activity typical of cookies and mimicking the effects of sodium chloride, ammonium hydrogen carbonate and sodium hydrogen carbonate in order to investigate the ability of spray dried OMWP to control acrylamide, HMF, FL, CML and CEL formation. The concentrations of 3-hydroxytyrosol, tyrosol, and verbascoside in the OMWP were 31 ± 0.29, 1.9 ± 0.11, and 2.8 ± 0.09 mg/g respectively. Oleuropein and other secoiridoids, including ligstroside, demethylcarboxyoleuropein and nüzhenide are the most abundant in raw OMW, while powdered ingredient used in this study showed a different composition as a consequence of the production process. Ingredient was obtained from OMW fractionated by using a filtration system at 37°C, then by spray drying process on the ultrafiltration retentate. Thermal loading favored the conversion of thermosensitive oleuropein into 3-hydroxytyrosol following previous evidence outlined by Jiménez *et al*. (2017). Acacia fiber and maltodextrin were used as coating agent for their chemical nature and encapsulation-related properties (low viscosity, emulsifying ability, solubility in water and low hygroscopicity) as detailed in Fang & Bhandari (2010). Indeed, the use of the carbohydrate mixture promoted the protection of secoiridoids and shaped the release of functional molecules during the thermal treatment, hence retarding the contact between aromatic ring and oxidizing agents and conditions typical of cookies during thermal treatment at 180 °C. This strategy was already shown in the frame of MR for controlled release of sodium chloride and ascorbic acid (Troise & Fogliano, 2013).

Figure 1 shows the evolution of HMF and acrylamide formation as well as the degradation of asparagine at 180 °C in a silica gel model system containing the buffering agents mentioned above: glucose, asparagine and 0.1% OMWP or a mixture of acacia fiber and maltodextrin without the

presence of secoiridoids derivatives. The concentration of asparagine and HMF was substantially similar in the three model systems up to 7 min; however, while after 5 min, acrylamide concentration in the presence of spray dried secoiridoids derivatives was significantly lower than the control model system without OMWP and the control in presence of acacia fiber and maltodextrin used as a coating agent. Acrylamide in the OMWP model system was significantly lower for all steps of the thermal treatment; in particular after 9 min, in the presence of OMWP, acrylamide was 32% lower than the control samples and the difference jumped to 47% for the control samples plus fibers after 13 min. Upon increasing the duration of the thermal treatment, protective effects of OMWP was also observed for HMF and asparagine, revealing significant differences when glucose and asparagine reacted in the presence of OMWP after 9 and 11 min with the sole exception of asparagine at 13 min.

Different results were obtained when free lysine reacted with glucose in the presence of spray dried ingredients and used as precursors of CML, CEL and FL formation (**Figure 2**). It was observed a significant reduction of the lysine in the presence of OMWP compared the two control samples from the beginning of the reaction process until 13 min, while no clear conclusion on FL can be drawn as the formation and degradation of the Amadori compound occurred before 5 min at 180°C. Surprisingly, the lowest CML concentration was observed in the control model system. Opposite trends were observed for CEL: the reduction in the presence of OMWP was up to 63% for the control after 9 and 11 min and up to 59% for the control plus fibers after 9 min. Taking into account the premises of silica model systems along with the color development as observed in previous papers, 13 min was used as a reference time for the study of acrylamide, d-AGEs and dicarbonyls formation in the cookies model systems (Fiore *et al*., 2012).

3.2. Cookies model systems.

Acacia fiber and maltodextrin in the silica model systems partly contributed to the Maillard pathways as in the case of CML. Upon the first screening in the silica environment, we decided to

test OMWP in cookies by using a lower concentration (0.05%) and a higher concentration (0.2%) compared to the silica model system. Following the standardized procedure for cookies preparation, three different concentrations, OMWP 500 (0.05%), OMWP 1000 (0.1%) and OMWP 2000 (0.2%) corresponding to 80, 160 and 320 mg were added to the recipe in order to test the effects of OMWP towards control cookies and control cookies plus 160 mg of a mixture (50:50, w/w) composed of acacia fiber and maltodextrin.

Figure 3 reports the results on dicarbonyls formation in five different model cookies thermally treated at 180 °C for 13 min. Independently of the OMWP amount, the concentration of glyoxal was reduced down to 80% compared to the control cookies, while the addition of the coating agent alone to the cookies lead to a reduction of glyoxal of around 30%. Synergistic effects were not as impressive as for methylglyoxal. In this case, the addition of acacia fiber and maltodextrin slightly increased the formation of methylglyoxal on the control cookies and these promoting effects influenced the concentration of methylglyoxal when spray dried powder was added. The final concentrations were 11.3, 8.7 and 12.1 ng/mg of cookies, for the three different levels of secoiridoids derivatives OMWP. No significant differences were obtained for the control of 2,3-butanedione formation. In all of the five cases, concentrations were close to 10 ng/mg of the samples. The results on the three dicarbonyls were in line with those previously reported by Degen *et al.* (2012), but 2,3-butanedione was one order of magnitude higher than the values reported in Kocadağlı *et al.* (2016). Looking at the presence of tyrosol and tyrosol derivatives, the reduction obtained here for glyoxal is close to those reported by Zhang *et al*. (2014) for cookies with flavonoids added. Results confirmed previous evidence on the ability of hydroxytyrosol and olive leaf extract in reducing dicarbonyl compounds as glyoxal and methylglyoxal: both compounds were in the same order of magnitude, even if in our case spray-dried ingredients were more effective than pure compounds or leaf extract (Navarro & Morales, 2017). **Figure 4** reports the concentration of furosine and d-AGEs as representative of lysine modifications. Furosine ranged from 7.78 to 12.4

ng/mg in the samples and the addition of acacia fiber and maltodextrin promoted the formation of Amadori compounds, with the consequent increase in all of four model systems toward the control recipe. The formation of CML was characterized by a similar trend as for furosine, while a significant reduction (down to 20% of CML) was achieved in the presence of 0.2% of OMWP toward control plus coating agents. The formation of CEL was characterized by different trends toward furosine and CML. A reduction was obtained in the presence of 0.05% OMWP while in the cases of the other samples, results were close to each other without a relevant impact of secoiridoids derivatives addition. Finally, the most interesting results were obtained for lysine. Taking into account that acacia fiber and maltodextrin were also able to protect lysine blockage, up to 88% of the lysine was blocked in the presence of 0.05 % OMWP, suggesting that other cross-links or end-products were influenced by the addition of OMWP. The results here reported are in line with other papers, taking into account specific differences in the recipe (Troise, Fiore, Colantuono, Kokkinidou, Peterson & Fogliano, 2014). Our results for CML matched those reported by Hull *et al*. for savory cookies (0.8–61.8 ng/mg foods) (Hull, Woodside, Ames & Cuskelly, 2012). In particular CML and CEL concentrations are in line with those reported by Scheijen *et al.* for cookies with multigrain or peanuts, but considering the addition of currants and cherries, the concentration of both CML and CEL was lower than those reported for cookies with 0.05% of OMWP (Scheijen *et al*., 2016). Furosine matched the concentration reported by Gökmen *et al*. for cookies with added sucrose thermally treated at 180°C (Gökmen, Serpen, Açar & Morales, 2008). **Figure 5** shows that concentrations of acrylamide and HMF ranged from 196.40 to 489.50 ng/g and from to 12.54 to 42.75 mg/kg, respectively. Acrylamide concentrations significantly increased in cookies with acacia fiber and maltodextrin toward control cookies and cookies added with OMWP at different concentrations. Specifically, in comparison to control cookies, the addition of 0.05% and 0.1% OMWP resulted in a reduction of acrylamide to 47% and 55%, respectively. Similarly, a decrease down to 54% and 60% was obtained when the same OMWP-rich samples were compared to control cookies plus coating agents. However, for 0.2% OMWP samples there were no

significant differences in acrylamide concentration when compared to the control cookies. Our results are in line with those reported by Zhu *et al*., and Li *et al*. that tested the effects of different plant extracts and polyphenols in inhibiting acrylamide formation in cookies (Li *et al*., 2012, Zhu, Cai, Ke & Corke, 2011). Furthermore, our results are in accordance with Arribas-Lorenzo *et al*., who measured a significant reduction of acrylamide levels in cookies by adding a polyphenol enriched olive oil to the formulation (Arribas-Lorenzo, Fogliano & Morales, 2009). The inhibiting effect of OMWP was also assessed toward HMF formation and our results demonstrated a greater effect of OMWP toward this intermediate. Specifically, HMF levels decreased in OMWP-rich cookies with a trend inversely proportional to the concentration of OMWP. Indeed, HMF was 76%, 58% and 35% compared to the control, when 0.05%, 0.1% and 0.2% OMWP were added in the cookie recipes, respectively. A similar trend was observed when OMWP-rich samples were compared to the control added with acacia fiber and maltodextrin. Our results are in accordance with Navarro and Morales, who demonstrated the inhibiting effects of hydroxytyrosol toward HMF formation in cookies (Navarro & Morales, 2017). Along with an overall decrease in intermediates and end-products formation, it worth mentioning the potential negative impact of polyphenols on taste and aroma attributes of final products. Polyphenols from OMWP are characterized by bitterness and astringency, in particular when added at high concentrations (De Toffoli *et al*., 2019).

The results obtained when an acacia fiber and maltodextrin mixture was added in cookies model systems can be explained local dehydration and or accumulation of reactants promoting a higher yield of the target analytes investigated. Indeed, water activity and reactants that transfer across different regions can be relevant contributors to the formation of glycation compounds. Meaningful examples can be observed in the case of Okara fiber (Palermo, Fiore & Fogliano, 2012), toasting processes of different kinds of flours, furfural, HMF and furosine content in whole and refined wheat flour (Rufián-Henares, Delgado-Andrade & Morales, 2009), in chitosan addition with opposite trends in case of HMF or acrylamide (Mogol & Gökmen, 2016) .

3.3 Chemical routes behind the control of MR

Figure 6 summarizes the potential pathways for the control of Maillard end-products and intermediates in the presence of OMWP. Starting from secoiridoids derivatives, we hypothesized and depicted the chemical routes able to potentially interfere with the formation of dicarbonyls, protein-bound d-AGEs and Amadori compounds, acrylamide and HMF. According to previous papers, we encompassed three key routes for the control of end-products and intermediates formation: trapping of dicarbonyls, antioxidant activity toward pro-oxidizing intermediates, and quinone formation before the reaction with free amino group. The ability of tyrosol, 3-hydroxytyrosol and verbascoside in controlling the formation of highly reactive dicarbonyls compounds has been extensively reviewed in particular from Morales and coworkers in different model systems (Navarro & Morales, 2017). Hypotheses about the ability of secoiridoids to trap dicarbonyls were firstly formulated by our group in milk systems, following evidences reported by Totlani and Peterson (2006) for catechins. In physiological conditions, Navarro *et al*. demonstrated that hydroxytyrosol, hydroxytyrosol-acetate, and 3,4-dihydroxyphenylacetic acid are able to scavenge C6 α-dicarbonyl compounds to a similar extent as methylglyoxal and glyoxal (Navarro, Atzenbeck, Pischetsrieder & Morales, 2016). We also postulated that tyrosol or 3-hydroxytyrosol undergo electrophilic aromatic substitution or they are able to delocalize resonance-stabilized phenoxy radicals, in the milieu of antioxidant mechanisms. According to Srey and coworkers, the addition of antioxidants can be an effective strategy to prevent the formation of CML and CEL in sponge cake (Srey, Hull, Connolly, Elliott, del Castillo & Ames, 2010). They observed that ferulic acid with a conjugated side chain in combination with a phenolic ring is able to exert an anti-CML/CEL effect attributed to its free radical scavenging activity, limiting the second phase of glycation supervised by glyoxal and methylglyoxal production. Conversely, the authors observed that α-tocopherol and rutin were scarcely able to suppress CML and CEL formation as a consequence of their partition coefficient and with their poor interaction aqueous soluble reactive

oxidation agents. In the present study, solubility of secoiridoids derivatives was optimized through the spray-drying process, and all three compounds investigated were able to effectively interact with CML formation and, to a certain extent, with lysine Amadori compound and CEL. The reduction of lysine bound glycation products can be a direct consequence of the reduction of glyoxal and methylglyoxal, but also a consequence of the direct reaction of the free epsilon amino group of lysine with oxidized quinone rings (Bittner, 2006). In this frame, three parallel mechanisms putatively led to the reaction of bound lysine with oxidized 3-hydroxytyrosol: Michael addition with the formation of *N*-quinonyl amino acids; imine formation including Schiff base formation and Strecker degradation with the release of aldehydes and 2-aminocarbonyl compounds. An oxidation step is required before each of these mechanisms, and both the presence of pro-oxidants arisen from lipids (Rizzi, 2006), and thermal treatment or intermediates of the Maillard reaction can promote oxidation of secoiridoids. Similar pathways have already been demonstrated for flavanols, and they extensively characterize such thermally treated products as tea, cocoa and coffee (Guerra & Yaylayan, 2014). Our hypothesis about free amino group-quinone interaction was further confirmed when moving from bound amino groups of lysine to free amino groups investigated in silica model systems with asparagine and free lysine. In the case of free lysine, we observed a faster decrease in the presence of OMWP supporting our hypothesis that steric hindrance of bulk protein can be a limiting factor in the reaction between secoiridoids and amino groups, while in the case of free asparagine in silica model systems the reduction was essentially the same with or without OMWP added. Similarly, to lysine, amino-quinone interactions were envisaged as the key mechanisms for the reduction of acrylamide in cookies and in silica model systems. Two contiguous routes were considered: the reaction of the α-amino group of asparagine with oxidized rings and acrylamide elimination upon the reaction with quinone according to the Michael addition (Koutsidis *et al*., 2009). Taking into account that hydroxytyrosol and oleuropein are potent scavengers of hydroxyl radicals (OH•), peroxynitrite (ONOOH), and superoxide radicals (O_2•$^-$) (Rietjens, Bast & Haenen, 2007), the stabilization of azomethine yilide can be another potential route for the control of

acrylamide formation (Yaylayan, Wnorowski & Perez Locas, 2003). The use of secoiridoids for the reduction of highly reactive amides is controversial: Kotsiou *et al*. showed that in emulsion model systems, aldehydic function may promote acrylamide formation confirming that partitioning and solubility of antioxidant compounds is not a secondary aspect (Kotsiou, Tasioula-Margari, Fiore, Gökmen & Fogliano, 2013). Conversely, Napolitano *et al*. observed that the concentration of dihydroxyphenolic compounds and acrylamide are inversely correlated in frying model systems (Napolitano, Morales, Sacchi & Fogliano, 2008, Sordini *et al*., 2019). Along with the oxidation into quinone, the formation of secoiridoids-lysine amide intermediates cannot be exclude as recently observed by Mertens and coworkers for hydroxycinnamic acids-lysine amide formation under mild conditions (Mertens, Heymann & Glomb, 2020). Regarding HMF in cookies samples, fructofuranosyl cation and 3-deoxyglucosone are the key intermediates and both can be influenced by the presence of phenolic compounds (Perez Locas & Yaylayan, 2008). As observed for acrylamide charged intermediates, cations formed during the synthesis of HMF can be stabilized by hydroxytyrosol, while for 3-deoxyglucosone a direct trapping of up to 43% was already demonstrated in physiological conditions (Navarro, Atzenbeck, Pischetsrieder & Morales, 2016).

4. Conclusions

In this paper, we demonstrated that OMWP is a versatile ingredient able to interfere at different stages of the MR. Due to the chemical nature of secoiridoids, multiple pathways promoted the control of lysine and asparagine modifications in a quaternary silica model system and in a more complex cookies model system. Specifically, in the silica model system the amount of acrylamide, lysine Amadori compound and CEL were reduced independently of the coating addition, while for CML reduction, secoiridoids were effective only in model systems with the addition of acacia fiber and maltodextrin as coating agents. In cookies, OMWP at three different concentrations significantly impacted the formation of protein bound Amadori compounds, CML, CEL, dicarbonyls, acrylamide and 5-hydroxymethylfurfural, highlighting the ability of secoiridoids based

functional ingredients in reducing the formation of d-AGEs. Finally, we hypothesized a major role for a spray-drying process that favored solubilization of OMW and promoted an effective interaction with water-soluble Maillard precursors and intermediates. Further studies will address the relationship between secoiridoids and Maillard taste active molecules development and the reactant location as an alternative strategy to control interplay between spray-dried functional molecules and Maillard precursors.

Note

The authors declare no competing financial interests, ADT and AC equally contributed to the manuscript.

Abbreviations used

CE, collision energy; MR, Maillard reaction; d-AGEs, dietary advanced glycation end-products; AP, Amadori product; HP, Heyns product; CML, *N*-ε-carboxymethyllysine; CEL, *N*-ε-carboxyethyllysine; FL, *N*-(1-deoxy-D-fructos-1-yl)-L-lysine; HMF, hydroxymethylfurfural; OMW, olive mill wastewater; OMWP, olive oil mill wastewater polyphenol powders; MRM, multiple reaction monitoring; LC-MS/MS, liquid chromatography tandem mass spectrometry; OPD, *o*-phenylendiamine, QX, quinoxaline, 2-MQX, 2-methylquinoxaline; 2,3-DMQX, 2,3-dimethylquinoxaline.

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References

Araújo, M., Pimentel, F. B., Alves, R. C., & Oliveira, M. B. P. (2015). Phenolic compounds from olive mill wastes: Health effects, analytical approach and application as food antioxidants. *Trends in Food Science & Technology*, 45(2), 200-211.

Arribas-Lorenzo, G., Fogliano, V., & Morales, F. (2009). Acrylamide formation in a cookie system as influenced by the oil phenol profile and degree of oxidation. *European Food Research and Technology; Zeitschrift für Lebensmittel- Untersuchung und -Forschung A*, 229(1), 63-72.

Bin, Q., Peterson, D. G., & Elias, R. J. (2012). Influence of phenolic compounds on the mechanisms of pyrazinium radical generation in the Maillard reaction. *Journal of Agricultural and Food Chemistry*, 60(21), 5482-5490.

Bittner, S. (2006). When quinones meet amino acids: chemical, physical and biological consequences. *Amino acids*, 30(3), 205-224.

De Marco, E., Savarese, M., Paduano, A., & Sacchi, R. (2007). Characterization and fractionation of phenolic compounds extracted from olive oil mill wastewaters. *Food Chemistry*, 104(2), 858-867.

De Toffoli, A., Monteleone, E., Bucalossi, G., Veneziani, G., Fia, G., Servili, M., Zanoni, B., Pagliarini, E., Toschi, T. G., & Dinnella, C. (2019). Sensory and chemical profile of a phenolic extract from olive mill waste waters in plant-base food with varied macro-composition. *Food Research International*, 119, 236-243.

Degen, J., Hellwig, M., & Henle, T. (2012). 1, 2-Dicarbonyl compounds in commonly consumed foods. *Journal of Agricultural and Food Chemistry*, 60(28), 7071-7079.

Delgado-Andrade, C., & Fogliano, V. (2018). Dietary advanced glycosylation end-products (dAGEs) and melanoidins formed through the Maillard reaction: physiological consequences of their intake. *Annual review of food science and technology*, 9, 271-291.

'EFSA' (2015). Scientific Opinion on acrylamide in food. *EFSA Journal*, 13(6), n/a-n/a.

Esposto, S., Taticchi, A., Di Maio, I., Urbani, S., Veneziani, G., Selvaggini, R., Sordini, B., & Servili, M. (2015). Effect of an olive phenolic extract on the quality of vegetable oils during frying. *Food Chemistry*, 176, 184-192.

Fang, Z., & Bhandari, B. (2010). Encapsulation of polyphenols–a review. *Trends in Food Science & Technology*, 21(10), 510-523.

Fiore, A., Troise, A. D., Atac Mogol, B., Roullier, V., Gourdon, A., El Mafadi Jian, S., Hamzalioglu, B. A., Gokmen, V., & Fogliano, V. (2012). Controlling the Maillard reaction by reactant encapsulation: sodium chloride in cookies. *Journal of Agricultural and Food Chemistry*, 60(43), 10808-10814.

Gökmen, V., Serpen, A., Açar, Ö Ç, & Morales, F. J. (2008). Significance of furosine as heat-induced marker in cookies. *Journal of cereal science*, 48(3), 843-847.

Guerra, P. V., & Yaylayan, V. A. (2014). Interaction of flavanols with amino acids: postoxidative reactivity of the B- ring of catechin with glycine. *Journal of Agricultural and Food Chemistry*, 62(17), 3831.

Hellwig, M., Gensberger-Reigl, S., Henle, T., & Pischetsrieder, M. (2018). Food-derived 1, 2-dicarbonyl compounds and their role in diseases. In *Seminars in cancer biology* (pp. 1-8). : Elsevier.

Hellwig, M., & Henle, T. (2014). Baking, ageing, diabetes: a short history of the Maillard reaction. *Angewandte Chemie International Edition*, 53(39), 10316-10329.

Hull, G. L., Woodside, J. V., Ames, J. M., & Cuskelly, G. J. (2012). Nε-(carboxymethyl) lysine content of foods commonly consumed in a Western style diet. *Food Chemistry*, 131(1), 170-174.

Jiménez, P., García, P., Bustamante, A., Barriga, A., & Robert, P. (2017). Thermal stability of oils added with avocado (Persea americana cv. Hass) or olive (Olea europaea cv. Arbequina) leaf extracts during the French potatoes frying. *Food Chemistry*, 221, 123-129.

Kocadağlı, T., Žilić, S., Taş, N. G., Vančetović, J., Dodig, D., & Gökmen, V. (2016). Formation of α-dicarbonyl compounds in cookies made from wheat, hull-less barley and colored corn and its relation with phenolic compounds, free amino acids and sugars. *European Food Research and Technology*, 242(1), 51-60.

Kotsiou, K., Tasioula-Margari, M., Fiore, A., Gökmen, V., & Fogliano, V. (2013). Acrylamide formation and colour development in low-fat baked potato products as influenced by baking conditions and oil type. *European Food Research and Technology*, 236(5), 843-851.

Koutsidis, G., Simons, S. P. J., Thong, Y. H., Haldoupis, Y., Mojica-Lazaro, J., Wedzicha, B. L., & Mottram, D. S. (2009). Investigations on the effect of amino acids on acrylamide, pyrazines, and Michael addition products in model systems. *Journal of Agricultural and Food Chemistry*, 57(19), 9011.

Li, D., Chen, Y., Zhang, Y., Lu, B., Jin, C., Wu, X., & Zhang, Y. (2012). Study on mitigation of acrylamide formation in cookies by 5 antioxidants. *Journal of Food Science*, 77(11), C1144-C1149.

Lund, M. N., & Ray, C. A. (2017). Control of Maillard reactions in foods: Strategies and chemical mechanisms. *Journal of Agricultural and Food Chemistry*, 65(23), 4537-4552.

Mertens, N., Heymann, T., & Glomb, M. A. (2020). Oxidative Fragmentation of Aspalathin Leads to the Formation of Dihydrocaffeic Acid and the Related Lysine Amide Adduct. *Journal of Agricultural and Food Chemistry*.

Mogol, B. A., & Gökmen, V. (2016). Effect of chitosan on the formation of acrylamide and hydroxymethylfurfural in model, biscuit and crust systems. *Food & function*, 7(8), 3431-3436.

Napolitano, A., Morales, F., Sacchi, R., & Fogliano, V. (2008). Relationship between Virgin Olive Oil Phenolic Compounds and Acrylamide Formation in Fried Crisps. *Journal of Agricultural and Food Chemistry; Relationship between Virgin Olive Oil Phenolic Compounds and Acrylamide Formation in Fried Crisps*, 56(6), 2034-2040.

Navarro, M., & Morales, F. J. (2017). Effect of hydroxytyrosol and olive leaf extract on 1, 2-dicarbonyl compounds, hydroxymethylfurfural and advanced glycation endproducts in a biscuit model. *Food Chemistry*, 217, 602-609.

Navarro, M., Atzenbeck, L., Pischetsrieder, M., & Morales, F. J. (2016). Investigations on the Reaction of C3 and C6 α-dicarbonyl compounds with hydroxytyrosol and related compounds under competitive conditions. *Journal of Agricultural and Food Chemistry*, 64(32), 6327-6332.

Navarro, M., Fiore, A., Fogliano, V., & Morales, F. J. (2015). Carbonyl trapping and antiglycative activities of olive oil mill wastewater. *Food & function*, 6(2), 574-583.

Nunes, M. A., Pimentel, F. B., Costa, A. S., Alves, R. C., & Oliveira, M. B. P. (2016). Olive by-products for functional and food applications: challenging opportunities to face environmental constraints. *Innovative Food Science & Emerging Technologies*, 35, 139-148.

Palermo, M., Fiore, A., & Fogliano, V. (2012). Okara promoted acrylamide and carboxymethyl-lysine formation in bakery products. *Journal of Agricultural and Food Chemistry*, 60(40), 10141-10146.

Perez Locas, C., & Yaylayan, V. A. (2008). Isotope labeling studies on the formation of 5-(hydroxymethyl)-2-furaldehyde (HMF) from sucrose by pyrolysis- GC/ MS. *Journal of Agricultural and Food Chemistry*, 56(15), 6717.

Rietjens, S. J., Bast, A., & Haenen, G. R. (2007). New insights into controversies on the antioxidant potential of the olive oil antioxidant hydroxytyrosol. *Journal of Agricultural and Food Chemistry*, 55(18), 7609-7614.

Rizzi, G. P. (2006). Formation of strecker aldehydes from polyphenol-derived quinones and α-amino acids in a nonenzymic model system. *Journal of Agricultural and Food Chemistry*, 54(5), 1893-1897.

Rufián-Henares, J. A., Delgado-Andrade, C., & Morales, F. J. (2009). Assessing the Maillard reaction development during the toasting process of common flours employed by the cereal products industry. *Food Chemistry*, 114(1), 93-99.

Scheijen, J. L., Clevers, E., Engelen, L., Dagnelie, P. C., Brouns, F., Stehouwer, C. D., & Schalkwijk, C. G. (2016). Analysis of advanced glycation endproducts in selected food items by ultra-performance liquid chromatography tandem mass spectrometry: Presentation of a dietary AGE database. *Food Chemistry*, 190, 1145-1150.

Sordini, B., Veneziani, G., Servili, M., Esposto, S., Selvaggini, R., Lorefice, A., & Taticchi, A. (2019). A quanti-qualitative study of a phenolic extract as a natural antioxidant in the frying processes. *Food Chemistry*, 279, 426-434.

Srey, C., Hull, G. L., Connolly, L., Elliott, C. T., del Castillo, M. D., & Ames, J. M. (2010). Effect of inhibitor compounds on N ε-(carboxymethyl) lysine (CML) and N ε-(carboxyethyl) lysine (CEL) formation in model foods. *Journal of Agricultural and Food Chemistry*, 58(22), 12036-12041.

Totlani, V. M., & Peterson, D. G. (2006). Epicatechin carbonyl- trapping reactions in aqueous Maillard systems: Identification and structural elucidation. *Journal of Agricultural and Food Chemistry*, 54(19), 7311-7318.

Troise, A. D., Wiltafsky, M., Fogliano, V., & Vitaglione, P. (2018). The quantification of free Amadori compounds and amino acids allows to model the bound Maillard reaction products formation in soybean products. *Food Chemistry*, 247, 29-38.

Troise, A. D., & Fogliano, V. (2013). Reactants encapsulation and Maillard reaction. *Trends in Food Science & Technology*, 33(1), 63-74.

Troise, A. D., Fiore, A., Colantuono, A., Kokkinidou, S., Peterson, D. G., & Fogliano, V. (2014). Effect of olive mill wastewater phenol compounds on reactive carbonyl species and maillard reaction end-products in ultrahigh-temperature-treated milk. *Journal of Agricultural and Food Chemistry*, 62(41), 10092-10100.

Troise, A. D., Fiore, A., & Fogliano, V. (2014). Quantitation of acrylamide in foods by high-resolution mass spectrometry. *Journal of Agricultural and Food Chemistry*, 62(1), 74.

Van Boekel, M. A. J. S. (1998). Effect of heating on Maillard reactions in milk. *Food Chemistry*, 62(4), 403-414.

Yaylayan, V. A., Wnorowski, A., & Perez Locas, C. (2003). Why asparagine needs carbohydrates to generate acrylamide. *Journal of Agricultural and Food Chemistry*, 51(6), 1753-1757.

Yeh, W., Hsia, S., Lee, W., & Wu, C. (2017). Polyphenols with antiglycation activity and mechanisms of action: A review of recent findings. *Journal of Food and Drug Analysis*, 25(1), 84-92.

Zamora, R., & Hidalgo, F. J. (2016). The triple defensive barrier of phenolic compounds against the lipid oxidation-induced damage in food products. *Trends in Food Science & Technology*, 54, 165-174.

Zhang, X., Chen, F., & Wang, M. (2014). Antioxidant and antiglycation activity of selected dietary polyphenols in a cookie model. *Journal of Agricultural and Food Chemistry*, 62(7), 1643-1648.

Zhu, F., Cai, Y., Ke, J., & Corke, H. (2011). Dietary plant materials reduce acrylamide formation in cookie and starch-based model systems. *Journal of the science of food and agriculture*, 91(13), 2477-2483.

Figure captions

Figure 1: asparagine decrease, acrylamide and HMF formation in silica model system treated at 180°C. Control sample with asparagine and glucose (CTRL), control reaction system in presence of olive oil mill wastewater polyphenol powders (OMWP), control sample in presence of acacia fiber and maltodextrin (ACFB).

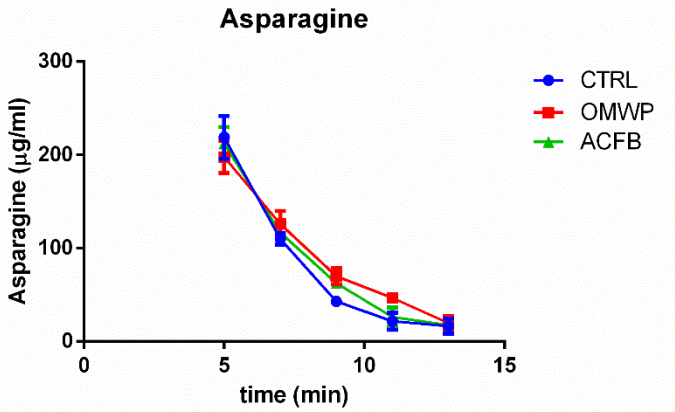
Figure 2: lysine decrease, Fru-Lys, CML and CEL formation in a silica model system treated at 180°C. Control sample with asparagine and glucose (CTRL), control reaction system in presence of olive oil mill wastewater polyphenol powders (OMWP), control sample in presence of acacia fiber and maltodextrin (ACFB).

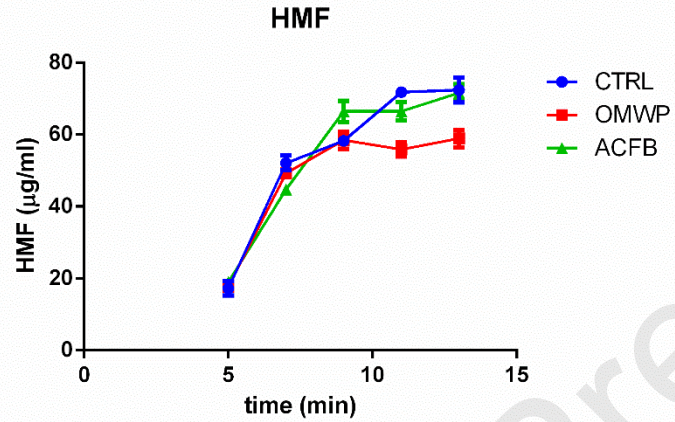
Figure 3: Formation of dicarbonyl compounds in cookies samples, treated at 180°C for 13 min. Control cookies (CTRL), control cookies with 0.05, 0.1 and 0.2% of olive oil mill wastewater polyphenol powders (OMWP500, OMWP1000, OMWP2000), cookies in presence of acacia fiber and maltodextrin coating (ACFB). Different letters correspond to significative differences (α, 0.05)

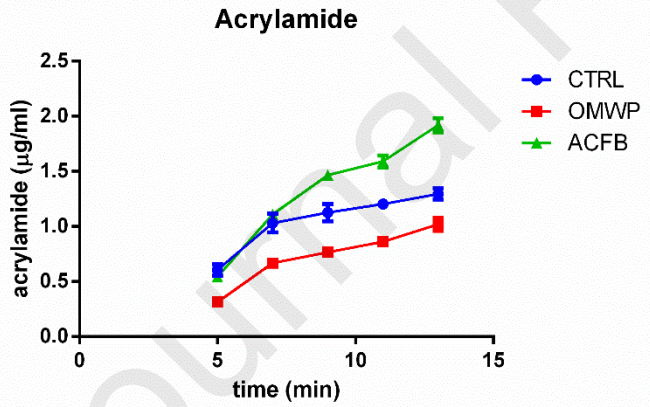
Figure 4: Lysine blockage and formation of furosine, CML and CEL in cookies treated at 180°C for 13 min. Control cookies (CTRL), control cookies with 0.05, 0.1 and 0.2% of olive oil mill wastewater polyphenols powder (OMWP500, OMWP1000, OMWP2000), cookies in presence of acacia fiber and maltodextrin coating (ACFB). Different letters correspond to significative differences (α, 0.05)

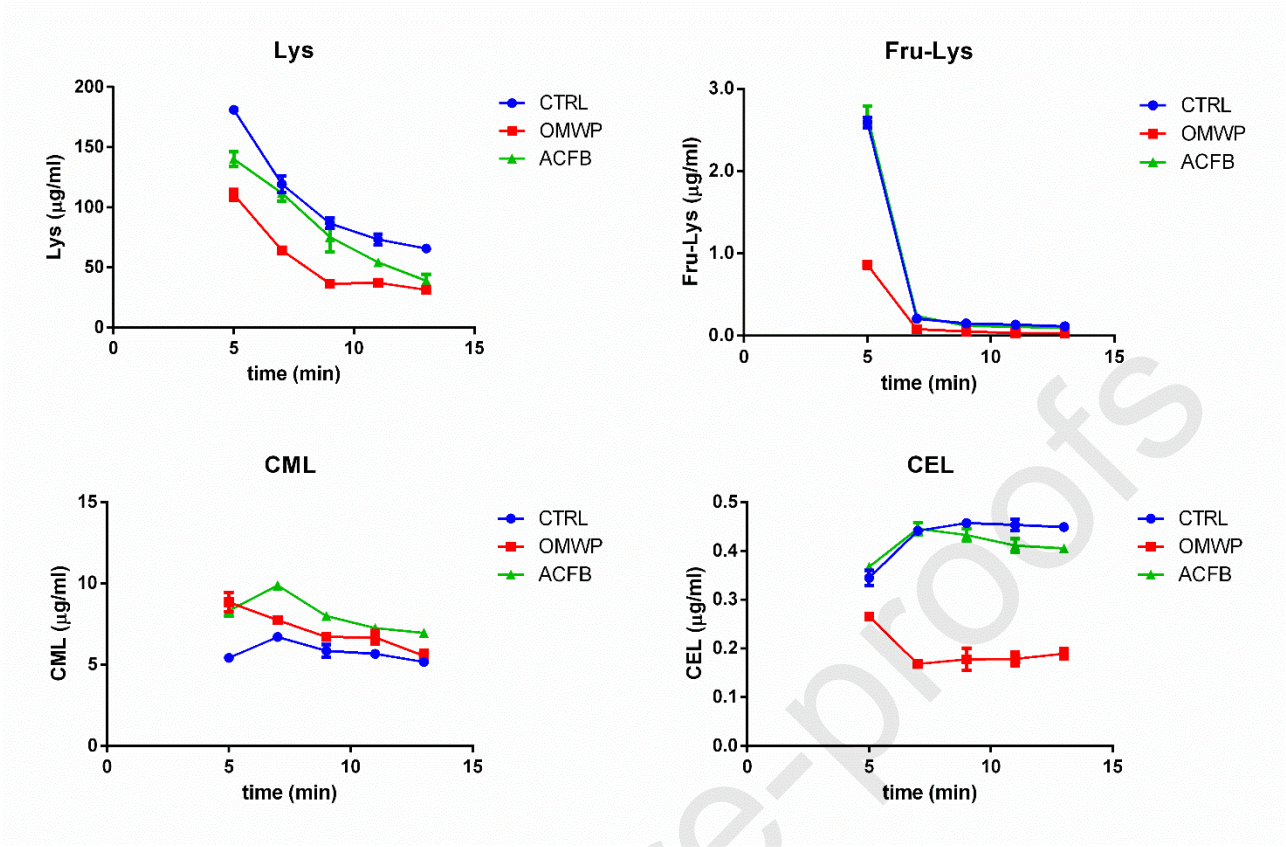
Figure 5: Formation of acrylamide and HMF in cookies samples, treated at 180°C for 13 min. Control cookies (CTRL), control cookies with 0.05, 0.1 and 0.2% of Olive oil mill wastewater polyphenol powders (OMWP500, OMWP1000, OMWP2000), cookies in presence of acacia fiber and maltodextrin coating (ACFB). Different letters correspond to significative differences (α, 0.05)

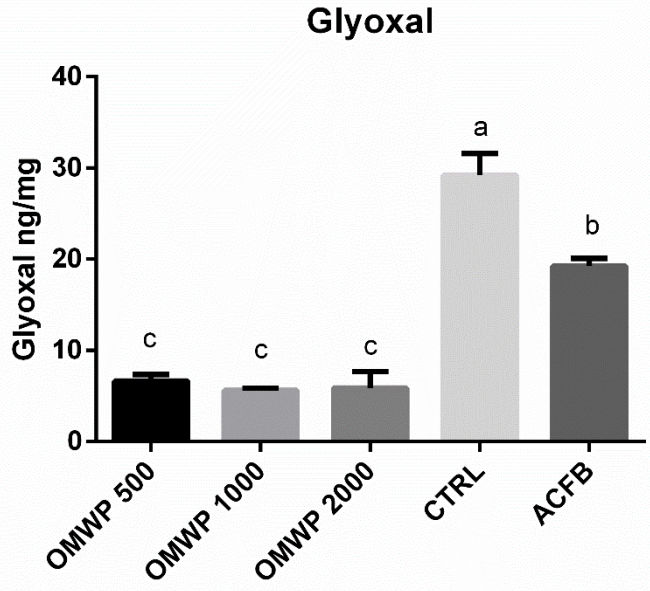
Figure 6: Reaction mechanisms behind the reduction of acrylamide (A), dicarbonyls (B) and d-AGEs (C). Each of the pathway includes Maillard reaction (red arrow) and interaction with secoiridoids derivatives. A-1, reaction between asparagine and oxidized secoiridoids through Schiff base and Michael addition, A-2: reaction between acrylamide and oxidized secoiridoids through Michael addition; B-1 and B-2: carbonyl trapping; C-1: reaction with free lysine through Schiff base formation and Michael addition; C-2: reaction with lysine bound to proteins. N.P. not present.

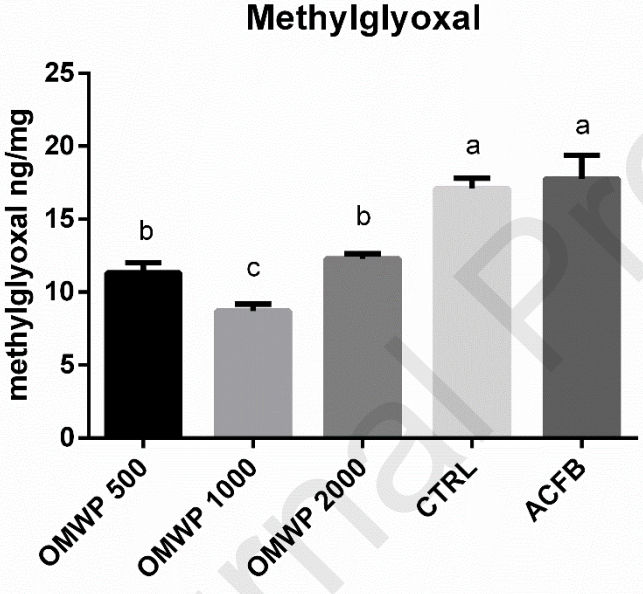


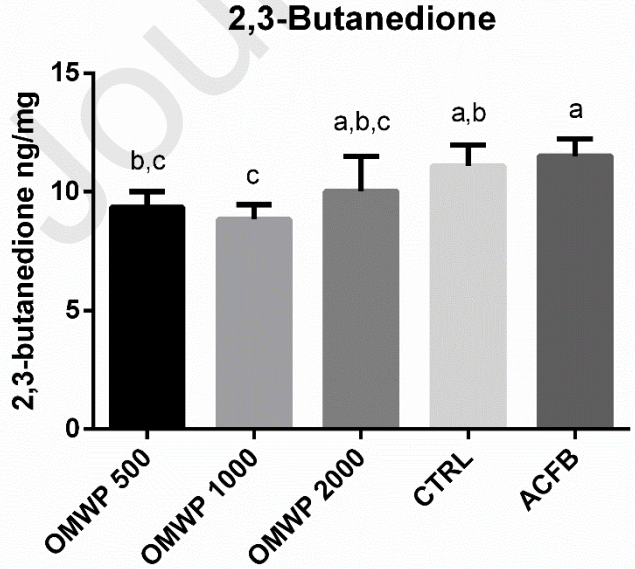


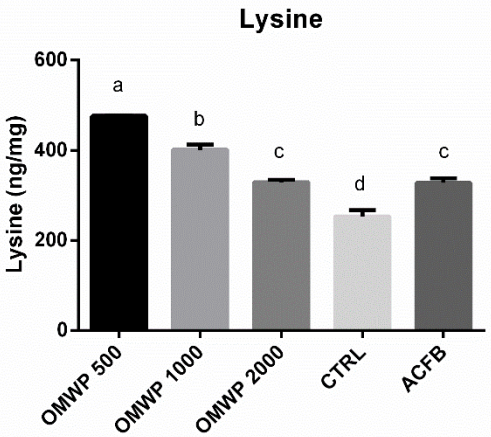


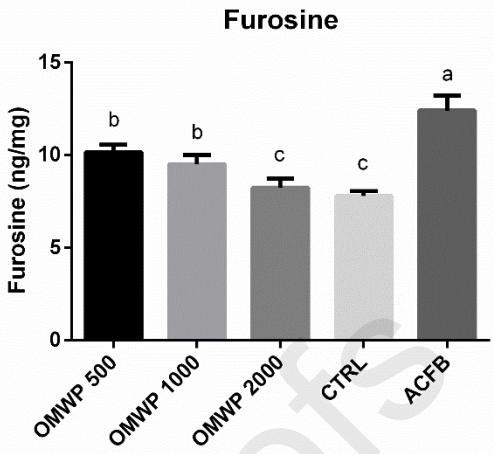


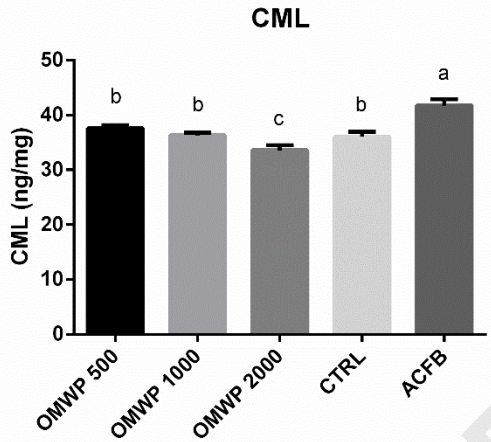


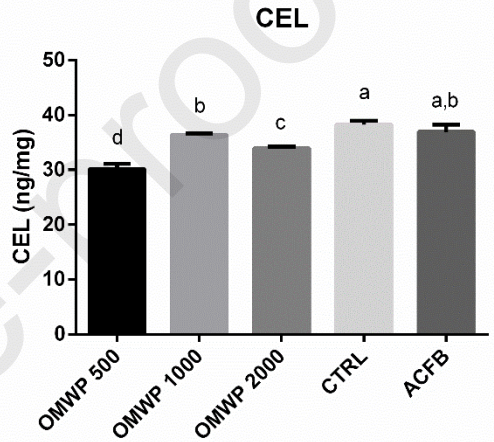


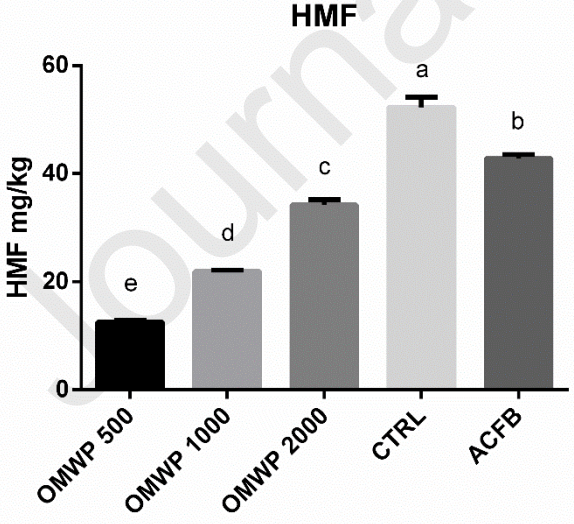


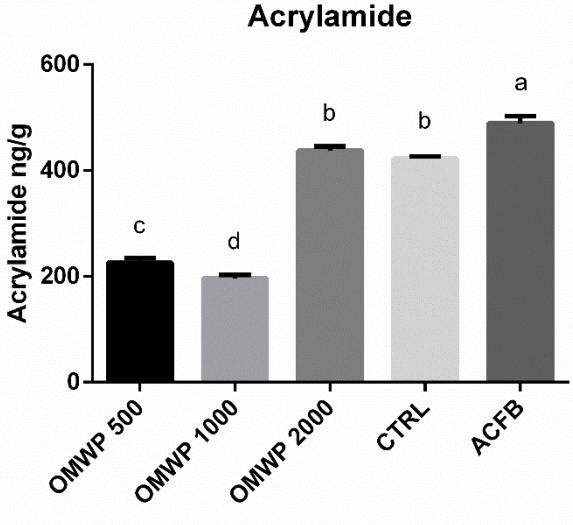


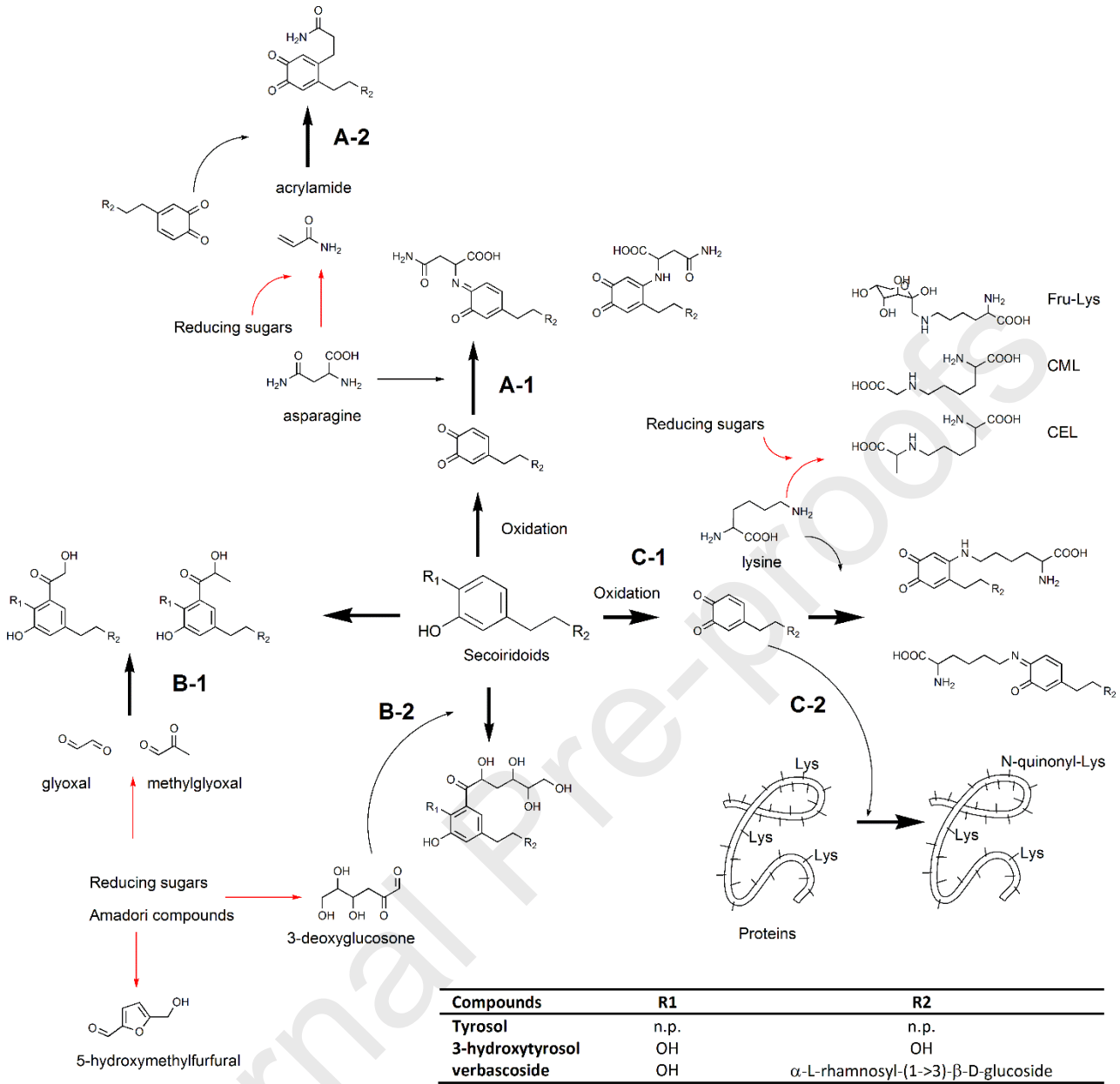










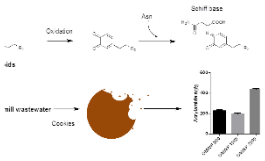


Compounds	R1	R2
Tyrosol	n.p.	n.p.
3-hydroxytyrosol	OH	OH
verbascoside	OH	α-L-rhamnosyl-(1->3)-β-D-glucoside

Supplementary Table: Cookies recipe, thermal treatment was performed at 180°C for 13 min, Control cookies (CTRL), control cookies with 0.05, 0.1 and 0.2% of Olive oil mill wastewater polyphenol powders (OMWP500, OMWP1000, OMWP2000), cookies in presence of acacia fiber and maltodextrin coating (ACFB). Shortening included palm oil. Weights are reported in grams (g)

Ingredients	Control	ACFB	OMWP2000	OMWP1000	OMWP500
Wheat flour	80	80	80	80	80
Sucrose	35	35	35	35	35
Shortening	20	20	20	20	20

Water	17.6	17.6	17.6	17.6	17.6
$NaHCO_3$	0.8	0.8	0.8	0.8	0.8
NaCl	1	1	1	1	1
NH_4HCO_3	0.4	0.4	0.4	0.4	0.4
OMWP	0	0	0.32	0.16	0.08
Acacia Fiber	0	0.08	0	0	0
Maltodextrin	0	0.08	0	0	0



Highlights

- A secoiridoids-rich ingredient was obtained from olive mill wastewaters
- 0.1% OMWP reduced acrylamide down to 47% and 55% in model system and in cookies
- OMWP significantly interfere at different stages of the Maillard Reaction
- Amino-quinone interactions are key mechanisms for acrylamide reduction

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Antonio Dario Troise: Investigation, Formal Analysis, Data Curation, Writing- Reviewing and Editing; **Antonio Colantuono**: Investigation, Formal Analysis, Data Curation, Writing- Original Draft Reviewing and Editing; **Alberto Fiore**: Conceptualisation, Methodology, Supervision, Data Curation, Writing- Reviewing and Editing.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: