Oleuropein as a bioactive constituent added in milk and yogurt

Evangelia Zoidou a,b, Prokopios Magiatis b,⁎, Eleni Mellou b, Maria Constantinou b, Serkos Haroutounian c, Alexios-Leandros Skaltounis b

a Laboratory of Dairy Research, Department of Food Science and Human Nutrition, Agricultural University of Athens, Iera Odos 75, Athens 11855, Greece
b Department of Pharmacognosy and Natural Products Chemistry, Faculty of Pharmacy, University of Athens, Panepistimiopolis, Zografou, Athens 15771, Greece
c Department of Animal Science and Aquaculture, Agricultural University of Athens, Iera Odos 75, Athens 11855, Greece

Abstract
Oleuropein is a bioactive natural product from olives known to display a broad variety of health beneficial properties. However, its presence in most edible olives is lowered due to debittering. In this respect, we envisaged the incorporation of oleuropein into dairy products (cow’s milk and yogurt) aiming to produce novel functional foods. Additionally, an analytical method for the monitoring of oleuropein in milk and yogurt was also developed and validated. Oleuropein was not affected during heat treatment of milk, while during the milk fermentation process it was not hydrolysed by the produced acids. Oleuropein was not metabolised by lactic acid bacteria, did not inhibit their growth and its stability in the final products was proven. The novel products displayed same taste, colour and texture as the conventional ones.

1. Introduction

Oleuropein (Fig. 1) comprises the major constituent of Olea europea leaves and unprocessed olives (Bianco & Uccella, 2000; Soler-Rivas, Espin, & Wichers, 2000). Many in vivo and in vitro studies have indicated that oleuropein exhibits a wide variety of biological activities, including antimicrobial, antioxidant, anti–ischaemic, antihypertensive, anticoagulant, hypolipidaemic and antcarcinogenic properties (Andreadou et al., 2006; Coni et al., 2000; Giamarellos-Bourboulis et al., 2006; Hamdi & Castellon, 2005; Obied, Malcolm, Danny, Paul, & Kevin, 2005; Tassou & Nychas, 1995). Thus, this natural product has attracted significant scientific and research interest, mainly connected with its potential protective role against infections and diseases, as well as the risk of developing breast, prostate and colon cancers, cardiovascular diseases and diabetes. Most significantly, according to the scientific opinion of EFSA (EFSA Journal, 2011) oleuropein (as hydroxytyrosol analogue) is related with the protection of the LDL from oxidation. The bioavailability of oleuropein has been recently studied (De Bock et al., 2013). It is absorbed after oral administration and is metabolised mainly to hydroxytyrosol which is also a powerful antioxidant.

Although oleuropein is an abundant constituent of unprocessed olives, in most edible olives it is removed during the debittering process, resulting in a significant decrease of its nutritional intake. Recently, we have identified a Greek edible olive variety (Throuba Thassos) which is particularly rich in oleuropein (Zoidou et al., 2010) and we determined an acceptable and safe level for the daily nutritional uptake of oleuropein. Based on these data, we envisaged the addition of oleuropein in dairy products (milk and yogurt) for the production of a new type of functional food which contain a quantity of oleuropein which approximates the amount of daily uptake from the aforementioned edible olive variety.

Milk and yogurt constitute two of the most popular dairy products with high nutritional value, which are proven as successful matrices for the development of various health-promoting functional foods (Rowan, Haggarty, & Ram, 2005). In recent years, a variety of dairy products supplemented with probiotic bacteria and/or bioactive components have been introduced to the market. Due to their health-promoting benefits, there has been an increasing interest in their development and consumption.

Oleuropein is available as a nutraceutical on the world market mainly as a constituent of olive leaf extract, but up today there are no food preparations containing this molecule. Since consumers demand for products combining the increased health benefits with desirable taste, the bitter taste of oleuropein constitutes a...
serious drawback to its use. The questionable stability of oleuropein during foodstuffs’ processing comprises a second barrier that has to be studied. Thus, the development of functional dairy products based on oleuropein is, therefore, an intriguing case.

In this endeavour, we present the production of milk and yogurt preparations containing various amounts of oleuropein. For this purpose, an extraction procedure and a chromatographic methodology for the determination of the presence and stability – during preparation and refrigerated storage – of oleuropein were developed and validated.

2. Materials and methods

2.1. Chemicals

All solvents and distilled water used throughout the experiments were obtained by Merck (Darmstadt, Germany) and were of HPLC grade. All mobile phases were vacuum filtered through a 0.2-μm membrane filter and degassed in an ultrasonic bath prior to HPLC analysis. Pure oleuropein was purchased from Extrasynthese (Genay, France).

2.2. Selection of oleuropein dosage

The determination of oleuropein dosage was based on a quantity that corresponds to the consumption of 15 olive drupes per day (as recently measured for Throuba Thassos olives (Zoidou et al., 2010)). For this purpose, oleuropein was added in milk to achieve three levels: 0.1 mg/ml, 0.2 mg/ml and 0.4 mg/ml. The organoleptic properties of the novel products were examined by a tasting panel which evaluated their taste, colour and texture using a 1–10 scale, in accordance with Tamine and Robinson (2000). The respective results suggested oleuropein concentrations of 0.1 mg/ml and 0.2 mg/ml were appropriate for further study.

2.3. Experimental procedure for the production of milk and yogurt preparations

Full-fat raw cow’s milk was used for all the experiments. Milk was first spiked with oleuropein and in a next step the content and stability of the contained oleuropein into milk and the produced yogurt were determined during their treatment. The experiment was accomplished in the laboratory in a batch process and afterwards on a pilot scale.

2.3.1. Milk processing

2.3.1.1. Heat treatment. The resistance of oleuropein during the heating of milk, as a routine practice for yogurt manufacture, was examined. In particular, 40 mg of pure oleuropein were added to 200 ml of raw milk (fat content 3.5%) and mixed. The milk was heated at 90 °C for 5 min, in a water bath under continuous stirring; then milk was cooled immediately with tap water and put into sterilised glass bottles. The oleuropein content was determined immediately after its addition to milk as well as after the heat treatment. The experiment was duplicated.

2.3.1.2. Storage at 4 ºC. Pure oleuropein (40 mg) was added into 200 ml of full fat commercial pasteurised cow’s milk (fat content 3.5%) and mixed well. Then, the milk was stored at 4 ºC for 7 days. The oleuropein content was determined at the beginning and every 2 days during storage. A blind sample containing milk was treated similarly, omitting the addition of oleuropein. The experiment was repeated twice.

2.3.2. Yogurt manufacture and storage

Pure oleuropein (90 mg) was added into 500 ml of full fat raw milk (fat content 3.5%), mixed well, heated at 90 ºC for 5 min in a water bath, cooled to 43 ºC and divided into equal quantities (2 × 200 ml) in two sterilised glass cups. Then, 3% (v/v) of yogurt culture was aseptically inoculated and mixed well and the milk was incubated at 42 ºC for 4.5 h, until its pH reached 4.45. The prepared yogurts were cooled, stored at 4 ºC and their oleuropein content was determined immediately after inoculation and the production of yogurt, as well as every 2 days during their storage. Control yogurt was also manufactured using a similar procedure without the oleuropein addition step. Preliminary experiments followed by sensory analysis were performed to determine the optimal dosage of oleuropein. The process was repeated twice in the laboratory and afterwards on a pilot scale using both dosages (0.1 mg/ml, 0.2 mg/ml).

2.4. pH measurement and sensory properties

During the refrigerated storage period, the quality of the produced milk and yogurt was monitored by measuring the pH and determining their sensory characteristics. In particular, pH was determined using a Hanna model HI 98240 pH meter (Hanna Instruments, Smithfield, RI), while the overall sensory acceptability of the new products was graded by a panel made up from the Dairy Laboratory staff. The products were served at 7–10 ºC, milk in glasses and yogurt in plastic cups, immediately after their preparation and after 7 and 35 days of storage at 4 ºC respectively. In this respect, their taste, colour and texture were graded using a sensory scale from 1 to 10, as follows: 1–2 bad, 3–4 not satisfying, 5–6 good, 7–8 very good, 9–10 excellent, according to Tamine and Robinson (2000). Their overall acceptability was also determined. Due to the small number in the sensory panel, the results should be considered as preliminary.

2.5. Quantitation of oleuropein in milk and yogurt

A method for the quantitation of oleuropein in milk and yogurt preparations was developed and validated. The proposed method included an extraction procedure of oleuropein and its quantitation through HPLC analysis.

2.5.1. Extraction procedure

The extraction procedure of milk was done with addition of 2 ml acetonitrile into 1 ml of milk under stirring for 2 min. Then the mixture was centrifuged at 2000 rpm for 15 min, the supernatant was filtered through a syringe filter and analysed by HPLC.

The extraction procedure of yogurt was as follows: 1 ml of water was added to 1 g of yogurt and stirred for 2 min. Then, the mixture was centrifuged at 2000 rpm for 15 min and the...
of colony-forming units (cfu) per ml or g. The enumeration of error Er%, defined as 100 divided by the concentration levels (0.02, 0.1 and 0.4 mg/ml or g) prepared on five replicates of milk and yogurt standards at three concentration levels were also determined along with the relative standard deviation % (RSD), accuracy, the relative percentage error % (Er) and determining the limits of detection and quantitation (LOD, LOQ). Moreover a reproducibility study (systematic percentage error % (Er) and determining the limits of detection and quantitation (LOD, LOQ) were determined by measuring the background response, and running six blank samples of milk and yogurt at maximum sensitivity. The signal-to-noise (S/N) ratio of 3:1 (peak area ratio of the oleuropein vs. baseline noise) and 10:1 were used for the calculation of the LOD and LOQ, respectively. The reproducibility study was performed by injecting a standard of 0.5 mg/ml in five replicates (n = 5).

2.7. LC–MS qualitative analysis

UHPLC–MS/MS monitoring of oleuropein was performed on an Agilent 1290 Infinity ultrahigh-pressure liquid chromatography system (UHPLC) interfaced to a 6460 triple-quadrupole mass spectrometer (QqQ MS/MS) with electrospray ionisation (ESI) via Jet Stream Technology (Agilent Technologies, Santa Clara, CA, USA). The UHPLC was equipped with a binary pump with an integrated vacuum degasser (G4220A), an autosampler (G4226A) with thermostat (G1330B), and a thermostated column compartment (G1316C). The samples were analysed using a Poroshell 120 EC-C18 column (2.1 × 150 mm 2.7 µm, Agilent Technologies). The mobile phase consisted of 0.1% formic acid in water (A) and acetonitrile (B) with the following gradient program: 0–2.5 min with 10% B, 3–6 min 25% B, 7.5 min 40% B, 8.5–9.5 min 95% B. The flow rate was 0.4 mL/min, and the injection volume was 1 µL. Negative ESI mode was used. The drying gas temperature and flow rate were 250 °C and 8 l/min, respectively. The sheath gas temperature and flow rate were 350 °C and 11 l/min, respectively. The nebuliser gas pressure, capillary voltage, and dwell time were 45 psi, 3.5 kV, and 200 ms, respectively.

Total ion as well as multiple reaction monitoring (MRM) mode was utilised to confirm the identity of oleuropein and the peak purity. Precursor and product ions were identified and optimised using MassHunter Optimizer (Agilent Technologies).

Oleuropein was monitored through the fragmentation of the precursor ion 539.2 to the product ion 275.1 using fragmentor voltage 165, collision energy 20 and retention time 6.3 min.

2.8. Microbiological analysis

The total viable microflora in milk and yogurt was enumerated by the pour-plate method using plate count agar (Merck, Darmstadt, Germany). The plates were incubated at 30 °C for 72 h (IDF 100B:1991) and microbiological count data was expressed as log10 colony-forming units (cfu) per ml or g. The enumeration was performed on milk or yogurt just after the inoculation with oleuropein, and then after 4 and 7 days for milk and after 14 and 35 days for yogurt at 4 °C. All determinations were made in duplicate.

2.9. Statistical analysis

Data were subjected to analysis of variance (ANOVA) using software Statgraphics Plus for Windows v. 5.2 (Manugistics, Inc., Rockville, MD) to test the effect of the concentration of oleuropein and storage time on yogurt characteristics.

3. Results and discussion

The selection of the appropriate dose for the oleuropein incorporation in the novel products constitutes a critical point for this endeavour. Based on the quantity of oleuropein measured for
Throuba Thassos variety (average 1.2 mg/drupe) (Zoidou et al., 2010) and considering that an intake of 15 olive drupes in a day is a usual olive consumption for the Greek population, the total oleuropein intake could be estimated as approximately 20 mg per day. In addition, since the average consumption of dairy products corresponds to 200 ml milk or 200 g yogurt per day, we selected three different levels of oleuropein 0.1, 0.2 and 0.4 mg/ml to incorporate into milk or yogurt (mg/g). These novel products were tested after their preparation by the sensory panel immediately, which found that the first two preparations exhibited acceptable sensory characteristics. On the contrary, milk and yogurt containing 0.4 mg/ml or mg/g of oleuropein tasted of rancid oil when swallowed and was only marginally acceptable.

3.1. Milk

3.1.1. Method validation

The method was evaluated through the determination of oleuropein content in milk revealing good linearity, precision, accuracy and reproducibility. The mean regression equation was \( y = 1153649x - 144663 \), with \( x \) accounting as the concentration and \( y \) the response (measured as peak area) values. All correlation coefficients calculated were higher than 0.990, ranging from 0.9955 to 0.9990. The data presented in Table 1, indicate that the proposed method can be considered as displaying adequate precision. In addition, the intra-day precision, expressed as RSD%, ranged from 3.7% to 7.9% for the three concentration levels and was low compared to the inter-day precision. The LOD and the LOQ were 0.025 mg/ml and 0.074 mg/ml, while the recovery in the milk samples was 73–93% (average 85%), indicative of a nearly quantitative recovery. The estimated accuracy values were within acceptable levels for oleuropein, while the system reproducibility was low (2.6%).

All milk control extracts used in the experiments were oleuropein-free, as determined by HPLC analysis.

3.1.2. Milk processing

According to quantitative HPLC-UV and qualitative LC–MS analysis, the oleuropein content in milk was not affected by heat processing, indicating the industrial application potential of this molecule in yogurt manufacture. The oleuropein stability was also tested during extended storage of milk at 4 °C, proving that the molecule remained intact. Furthermore, no metabolites were detected at any screened UV wavelength, indicating that no decomposition of this compound occurred. It must be noted that according to previous reports and patents the addition of some phenolics into milk prior to heat treatment enhanced its storage stability (Morgan, Anderson, & Hankinson, 1971). In addition, according to O’Connell and Fox (2001), the ability of various phenols to improve milk processability is attributed to their interaction with milk proteins. In particular, Sarker, Wilde, and Clark (1995) reported that catechin, a green tea polyphenol, increases the volume and improves the foaming properties of β-lactoglobulin, while phenol-rich extracts or purified phenols such as caffeic acid, markedly increase the heat stability of milk at 140 °C (O’Connell & Fox, 1999).

3.2. Yogurt

3.2.1. Method validation

The HPLC-UV method was evaluated through the determination of oleuropein in the novel yogurt preparation, displaying a linear relationship between oleuropein response (measured as peak area) and the corresponding concentration. The equation describing this relationship was \( y = 10490596x - 71707 \), where \( x \) is the concentration and \( y \) the response value. The correlation coefficients were higher than 0.990, ranging from 0.997 to 0.9990. The data presented in Table 1 reveal good precision and accuracy, with the lowest values corresponding to the lower quantities of oleuropein (0.02 mg/g). The limits of detection (LOD) and quantitation (LOQ) were 0.003 mg/g and 0.009 mg/g respectively, while the recovery in the yogurt samples was close to 75%, the lower value obtained at the lower concentration.

All yogurt control extracts used in the experiments were oleuropein-free, as determined by HPLC and LC–MS analysis.

3.2.2. Yogurt manufacture and storage

The fermentation period of the milk supplemented with oleuropein was found to be normal (2.45 h), a value comparable to the control milk. Though oleuropein is considered as an antimicrobial agent, herein it did not inhibit the growth of lactic acid bacteria, when they were incubated together. In previous studies it has been reported that oleuropein has an antimicrobial activity against brine lactic acid bacteria Lactobacillus plantarum, Lactobacillus brevis, Leuconostoc mesenteroides, Pediococcus cerevisiae (Fleming, Walter, & Etchells, 1973). However, no data exist about the ability of oleuropein to inhibit the growth of yogurt bacteria. According to HPLC results, the concentration of oleuropein determined by HPLC after 4.5 h incorporation in yogurt was similar to that in the processed milk. Fermentation for several hours does not cause any degradation to oleuropein, suggesting that this molecule is stable to hydrolysis by starter bacteria. It must be noted that Giafardini, Marsilio, Lanza, and Pozzi (1994), and Marsilio and Lanza (1998), studied the ability of an oleuropein degrading strain of L. plantarum, to grow in the presence of oleuropein. They found out that in the absence of glucose, the oleuropein incorporation in the cultivation medium caused complete degradation to derivative products, whereas in the presence of glucose oleuropein remained almost intact in the cultivation medium. This observation indicates that oleuropein is degraded unless there is another carbon and energy source – as glucose – which may be utilised more readily. McCue and Shetty (2005) also demonstrated that other phenolics were also not affected by yogurt microflora in the presence of kefir culture during yogurt production from soymilk.

Moreover, the acids produced during lactic acid fermentation did not affect oleuropein. After 4.5 h of storage the pH of yogurt containing oleuropein was 4.45, a value comparable to the control, indicating that oleuropein neither supported nor impeded lactic acid production. It is known that iridoids are sensitive in acids or bases, however seco-iridoids like oleuropein are considered as more stable.

### Table 1

<table>
<thead>
<tr>
<th>Oleuropein</th>
<th>Intra-day precision RSD% (n = 5)</th>
<th>Inter-day precision RSD% (n = 3)</th>
<th>Accuracy Er% (n = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Milk</td>
<td>Yogurt</td>
<td>Milk</td>
</tr>
<tr>
<td>0.02</td>
<td>7.9</td>
<td>7.1</td>
<td>8.6</td>
</tr>
<tr>
<td>0.10</td>
<td>3.9</td>
<td>4.5</td>
<td>5.4</td>
</tr>
<tr>
<td>0.40</td>
<td>3.7</td>
<td>5.6</td>
<td>6.9</td>
</tr>
<tr>
<td></td>
<td>Milk</td>
<td>Yogurt</td>
<td>Milk</td>
</tr>
<tr>
<td>0.02</td>
<td></td>
<td></td>
<td>-9.6</td>
</tr>
<tr>
<td>0.10</td>
<td></td>
<td></td>
<td>-4.1</td>
</tr>
<tr>
<td>0.40</td>
<td></td>
<td></td>
<td>0.7</td>
</tr>
</tbody>
</table>
The stability of oleuropein was also tested during extended storage of yogurt at 4 °C. As shown in Fig. 2, oleuropein was found to be chemically stable in acidic conditions up to 35 days of storage (a slight reduction after the 27th day was not considered as statistically important). The chemical stability was also confirmed using qualitative LC–MS analysis.

Concerning the bacterial population of the manufactured milk and yogurt during storage, the following data were collected: The average total viable microflora in raw milk was 4.78 log cfu/ml. After the heat treatment it was 2.20 log cfu/ml and after 4 days and 7 days storage at 4 °C was 3.45 and 4.64 log cfu/ml, respectively. Viable microflora in the 1st day yogurts was 8.71 log cfu/g. After 14 days the average counts dropped to 8.33 and at the end of the refrigerated storage to 7.52 log cfu/g (Supplementary Table 1). All yoghurt samples, however, contained 10⁷–10⁸ cfu/g for the whole period of 35 days. In general, there were not significant differences for total microflora among milk or yogurt samples with or without oleuropein.

3.3. pH measurements and sensory characteristics

After manufacture and storage, the quality of the two novel dairy products was evaluated by determining their pH values and sensory characteristics. With respect to the pH, a similar pattern was observed between the test and the control products, since the pH value was 6.45 in milk and was not markedly changed after storage for 7 days at 4 °C. In the case of fresh yogurts the pH was measured at 4.45 and diminished from 4.29 to 4.24, after 15 and 35 days of storage.

### Table 2

Sensory analysis of yogurts incorporated with oleuropein during storage at 4 °C.

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Storage period (days)</th>
<th>Yogurt code</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>A</td>
</tr>
<tr>
<td>Texture</td>
<td>1</td>
<td>8.01&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>8.33&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>8.70&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Taste</td>
<td>1</td>
<td>7.11&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>7.07&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>6.18&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Colour</td>
<td>1</td>
<td>8.52&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>8.29&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>8.04&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total</td>
<td>1</td>
<td>23.64&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>23.69&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>35</td>
<td>22.92&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

A: Yogurt with 0.1 and 0.2 mg/g oleuropein respectively; C: yogurt without oleuropein.

*Means in row at the same storage time with a common superscript do not differ significantly (p > 0.05, LSD test).

<sup>a</sup>Means in column at different storage time with a common superscript do not differ significantly (p > 0.05, LSD test).
The sensory acceptability of the novel milk and yogurt was also tested immediately after their preparation and after 7 and 35 days of storage. The two selected concentration levels of oleuropein (0.1 mg/ml and 0.2 mg/ml or g) gave preparations equally acceptable with the controls (p > 0.05), since changes in yogurt taste were not observed (Table 2). Many researchers have reported that the addition of phenolic compounds into dairy products alters their organoleptic properties. In some cases phenols were also responsible for distinct off-flavours, due to proteins interaction through Maillard reactions (Luck et al., 1994; Parks & Allen, 1973; Walker & Manning, 1976) or oxidation, after the heat treatment of milk (Dumont, Roger, Cerf, & Adda, 1974) or even when they were added as flavouring agents (Maga, 1988). Thus, the effect of phenols as functional ingredients on the quality of dairy products has been advocated and has been attributed to protein–polysaccharide–phenols interactions. The extent of this interaction depends on the pH, the molecular properties of phenols or the presence of specific polysaccharides. The enzymatic oxidation to quinones may also play an important role as well (O’Connell & Fox, 2001). Finally, the sensory scores of milk and yogurt prepared for the experiment, after 7 days and 35 days of storage respectively, were also determined. It should be noted that the mean scores for taste, and colour gradually decreased, while the mean scores for texture increased in yogurts as the storage progressed.

Generally, the oleuropein-based yogurts were firmer than control yogurts. However this difference was not significant, indicating that the lactoglobulin–casein complex was not affected by the presence of oleuropein.

These changes in sensory characteristics were similar with the control samples. Nevertheless, all the products were acceptable to the sensory panel, characterised as ‘very good’ and none of them had any off flavour.

4. Conclusion

The present work is considered as the first report concerning the addition of oleuropein in milk and yogurt to produce novel foodstuff preparations. For this purpose, two efficient methods have been developed and validated for the reliable determination of oleuropein in milk and yogurt preparations. The results herein indicated that oleuropein is resistant during heating of milk. During coagulation of milk, oleuropein was not hydrolysed by the produced acids, it was not metabolised by lactic acid bacteria and did not inhibit their growth. Oleuropein was completely soluble in the selected concentrations (0.1 mg/ml or 0.2 mg/ml) without adding any peculiar taste or flavour, while its stability during the milk and yogurt storage at 4 ºC was unequivocally proven. Since from the technological point of view, the presence of oleuropein does not interfere with milk and yogurt manufacturing process and considering the significant biological value of oleuropein, proved repetitively by numerous research reports, it is concluded that this molecule can be added as an active ingredient in milk and yogurt preparations for the production of novel functional foods with significant health benefits. This finding also implies that olive extracts preparations for the production of novel functional foods with significant health benefits. This finding also implies that olive extracts

opportunity to E.M. to run LCMS experiments in the Food Science Department of the University of California, Davis.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2014.02.137.

References


EFS A (2011). Scientific Opinion on the substantiation of health claims related to polyphenols in olive and protection of LDL particles from oxidative damage. EFSA Journal, 9, 2033.


