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Reduction of acrylamide content of ripe olives by selected additives

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ABSTRACT

A model system based on alkali-treated olive juice heated at 121 °C for 30 min was used to screen different additives (salts, amino acids, antioxidants) for potential inhibition of acrylamide formation in ripe olives. The most-efficient inhibitors found were sodium bisulphite, L-cysteine, and L-arginine. These compounds, as well as other sulphur-containing compounds (*N*-acetyl-L-cysteine, reduced glutathione, methionine) and several natural products (tea, oregano, rosemary, garlic), were then added to black ripe olives prior to sterilisation to evaluate their effect on both the acrylamide content and the sensory quality of olives. Sodium bisulphite had the highest impact on the acrylamide level in black ripe olives without a negative repercussion on sensory quality. Arginine and blanched garlic showed promising results. SH-containing compounds such as L-cysteine, *N*-acetyl-L-cysteine, or reduced glutathione were as effective as sodium bisulphite in reducing acrylamide, but did generate unpleasant off-flavours.

1. Introduction

Acrylamide (AA), which has been classified as probably carcinogenic to humans, has been found in many foods (Friedman, 2003). Recently, exploratory surveys in foods performed by the US. FDA have shown that canned black ripe olives, a product for which a sterilisation treatment is mandatory, contained considerable levels of AA (FDA, 2006). More recently still Casado and Montaño (2008) found a wide range (176–1578 μg/kg) for AA content in this product, suggesting that there may be ways to decrease the amount of AA by optimising the processing conditions. It has been demonstrated that AA in olives is formed during the sterilisation treatment (Amrein, Andres, Escher, & Amadó, 2007). Montaño, Casado, Rejano, and Sánchez (2008) studied the influence of sterilisation temperature and time on AA content in packed alkali-treated olives. It was demonstrated that: (1) for temperatures between 110 and 125 °C the AA content increased significantly ($p < 0.05$) with increasing time, and (2) for equivalent heat treatments (i.e. processes with the same F_0 value) the AA content decreased with increasing temperature. The influence of different processing conditions, including preservation method, darkening method, olive cultivar, and presentation form, on AA content in black ripe olives has been investigated by Casado and Montaño (2008).

Data from the above-mentioned studies suggested some ways to decrease the amount of AA in ripe olives: (i) by selecting the most suitable olive cultivar (Hojiblanca better than Manzanilla), (ii) by using a darkening method with two water washings better than with one water washing, and (iii) by strict attention to sterilisation conditions (the minimum value for accumulated lethality of $15F_0$ should be reached by using the highest sterilisation temperature, and such value should not be exceeded by much).

Another strategy to mitigate the formation of AA in foods – the use of additives – has not been investigated in ripe olives. Mestdagh et al. (2005) studied the impact of various additives on the formation of AA in a potato model system, using a heating method based on a closed stainless steel tubular reactor. A similar tool was used by Claeys, De Vleeschouwer, and Hendrickx (2005) to study the effect of amino acids in an asparagine–glucose model system. Knowing the effect of these additives on AA formation could provide useful information on the reaction mechanism. As previously found (Casado & Montaño, 2008), free asparagine and reducing sugars are not important AA precursors in ripe olives, in contrast to the case of other cooked foods (e.g. fried potatoes, bread, roasted almonds, roasted tea).

In the present work, a model system based on juice from alkali-treated green olives, which approximated the chemical composition of green ripe olives before sterilisation, was used to study the pH effect and to screen different additives for potential inhibition of AA formation in ripe olives. The most-efficient additives, as well as different natural products, were then evaluated in the actual product (black ripe olives), taking into account their impact on both AA content and sensory quality.

2. Materials and methods

2.1. Reagents, chemicals, and natural products

All reagents and chemicals used for the acrylamide analysis were as described in Casado and Montaño (2008). Inorganic salts,

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individual amino acids, and compounds such as *N*-acetyl-L-cysteine (NAC), reduced glutathione (GSH), and propyl gallate were supplied by Sigma–Aldrich (St. Louis, MO). Deionised water (Milli-Q; Millipore Corp.) was used throughout. Hydroxytyrosol (HT) and 3,4-dihydroxyphenyl glycol (DHPG) were a gift from Dr. Fernandez-Bolaños (Instituto de la Grasa, Seville, Spain). All other chemicals and solvents were of analytical or chromatographic grade from various suppliers. Natural products were purchased in a local market. Green tea was purchased as tea bags (Java green tea, Twinings, London), and oregano and rosemary were purchased as bottled dried herbs (Carmencita, Alicante, Spain). Garlic bulbs of Spanish origin were of "purple garlic" type. Blanched garlic was prepared as follows: garlic cloves of three bulbs were peeled and heated in 1 l of boiling distilled water for 3 min. Before being used in the experiments, raw or blanched garlic cloves were minced with a hand blender.

2.2. Effect of pH and selected additives in olive model system

For olive juice preparation, olives (Hojiblanca cultivar) were sorted, and about 5 kg were treated in a cylindrical container with 4.1 l of lye (2.2% w/v NaOH) for 6 h at room temperature. This solution was removed, and the fruits were washed with 4.1 l of tap water for 3 h. The washing stage was repeated, but with a distinct duration (15 h), and then the olives were pitted and washed once more for 6 h. Finally, the olives were homogenised using a mixer, and the juice was obtained by filtration through cheesecloth and centrifugation of the filtrate at 20,000*g* for 15 min (to remove oil). The juice was stored at −30 °C until analysis and heat treatment. At that moment, the juice had a pH value of 8.16, which is a value normally found in the flesh of ripe olives prior to sterilisation treatment (García, Brenes, & Garrido, 1994).

For the study of the effect of pH on acrylamide formation, the pH of the juice was adjusted by addition of 6 N HCl or 2 N NaOH, using a pH electrode (Crison Instruments, Barcelona, Spain), in a pH range between 4 and 9, and then submitted to heat treatment.

For the study of the effect of additives on acrylamide formation, selected salts, amino acids, and antioxidant compounds were added separately to juice in a known concentration. In all cases, prior to heating, the mixture pH was adjusted to 7 to rule out acrylamide mitigation due to a pH effect.

Heat treatment was performed by placing olive juice (5 ml) in a custom-made stainless steel tubular reactor (internal diameter 1 cm, length 8.5 cm). The reactor was sealed, and then heated in a thermostatted oil bath at 121 °C (±1 °C) for 30 min. After heating, the sample was immediately cooled in ice water for 3 min to stop any further reaction. Finally, the juice was analysed for its acrylamide content. All experiments were performed in duplicate.

2.3. Effect of additives and natural products on black ripe olives

Pitted black ripe olives (Hojiblanca cultivar), without sterilisation treatment, were supplied by a local olive processor. Fruits were packed in "A314" glass bottles (145 g of pitted olives plus 170 ml of brine capacity) and covered with brine containing 3% NaCl, 0.015% ferrous gluconate, and the corresponding compound or natural product ($NaHSO_3$, $CaCl_2$, Cys, Arg, Met, NAC, GSH, propyl gallate, oregano, rosemary, tea, or garlic). Compounds were added to give a fixed equilibrium value (50 mM, in general), while natural products were added at the level of 1 g/bottle, with the exception of blanched garlic, which was tested at two levels (5 g/bottle and 25 g/bottle). A control product was prepared using the same brine without any additive. If necessary, before the olives were covered, the pH of the packing brine was adjusted to 6.5–7.0 by addition of NaOH or HCl. Bottles were stored at 4 °C for 45 h prior to the sterilisation treatment to allow equilibration of the packing brine with the olives. Three bottles from each treatment were heated at 121 °C for 15 min in a computer-controlled retort (Steriflow, SAS, Paris, France). Sterilised bottles were stored at room temperature for one month before determination of acrylamide content along with physicochemical and sensory characteristics.

2.4. Analysis of acrylamide

The AA content was determined by a gas chromatography–mass spectrometry (GC–MS) method (with previous derivatisation of AA), as described previously (Casado & Montaño, 2008). After addition of ($^{13}C_3$)acrylamide as an internal standard and further clean-up of the sample, bromination of the acrylamide double bond was performed. The reaction product (2,3-dibromopropionamide) was extracted with ethyl acetate and dried over sodium sulphate, and the solvent was evaporated to dryness under a stream of nitrogen. The derivative was then converted to 2-bromopropenamide by dehydrobromination with triethylamine, and analysed by GC–MS.

2.5. Physicochemical and sensory characteristics

The analyses of olive brines for pH, titratable acidity (expressed as % lactic acid), combined acidity (expressed as normality, i.e. equivalents of HCl added to 1 l of brine to reach pH 2.6), and salt content (expressed as % NaCl) were carried out using the routine methods described elsewhere (Garrido, Fernández-Díez, & Adams, 1997).

The firmness of olives was measured using a Kramer shear compression cell coupled to an Instron test device (Model 1011). The cross-head speed was 200 mm/min. The firmness of the olives in each bottle was expressed as the mean of 10 replicated measurements, each of which was performed on four olives. Shear compression force was expressed as N/g.

Colourimetric measurements on the olives were made using a Colour-View Model 9000 spectrophotometer (BYK-Gardner, Inc., Silver Spring, MD). Interference by stray light was minimised by covering the samples with a box containing a matt black interior. Colour was expressed as reflectance at 700 nm (R_{700}). Lower reflectance values indicate darker colours. The data of each measurement are the average of 20 olives.

Product flavour was evaluated by an 11-member sensory panel. Evaluations were done in individual sensory booths under controlled environmental conditions (20–22 °C, incandescent lighting). Paired comparison tests were performed in order to determine whether samples with selected additives were significantly different from a control sample (without additive).

2.6. Statistical analysis

The general regression model of software Statistica version 7.0 (Statsoft Inc., Tulsa, OK) was used to test the effect of pH on acrylamide formation (Fig. 1). The 95% confidence intervals in Figs. 2–4 were calculated assuming that the coefficient of variation in heating experiments was 6%. Sensory data from the paired comparison tests were compared with tabulated values, taking into account the number of judges and a confidence level of 95% (Ordóñez-Aranguren, 2001, chap. 7).

3. Results and discussion

3.1. Influence of pH on acrylamide formation in olive juice

For the juice heated at 121 °C for 30 min, the curve of acrylamide content as a function of pH exhibited a maximum between pH 5.5 and 6.0 (Fig. 1), which is lower than in studies with

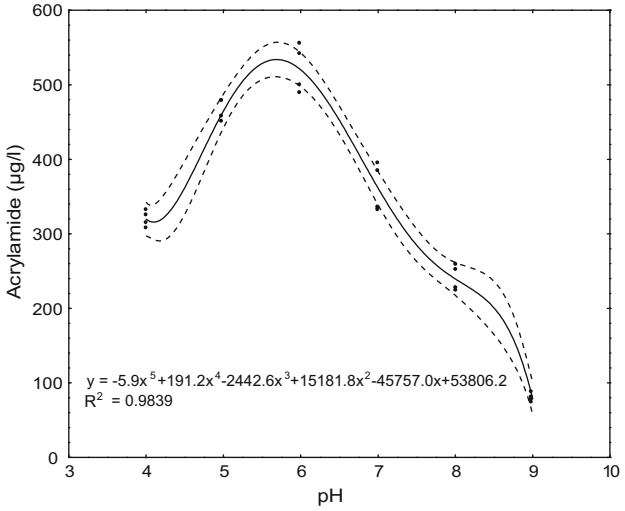

Fig. 1. Effect of pH on the content of acrylamide in olive juice heated at 121 °C for 30 min. Solid line: fitted equation; dashed lines: 95% confidence intervals on the equation.

homogenised potato (optimum pH around 8) (Rydberg, Eriksson, Tareke, Karlsson, & Ehrenberg, 2003) or in a potato model system (optimum pH between pH 7 and 7.5) (Mestdagh et al., 2008). From these heating experiments, the repeatability of the heating procedure was estimated by averaging the coefficients of variation (CV) of acrylamide content at each pH value, which gave a CV of 6% ($n = 6$). This value is only slightly higher than the precision (repeatability) of the analytical method for acrylamide, with a CV of 2.9% (Casado & Montaño, 2008). A fifth-order mathematical model showed the best fit ($R^2 = 0.9839$). The pH curve indicates that acrylamide formation is significantly reduced at pH values above 8 or below 5. However, in the actual product (ripe olives), these pH values have been demonstrated to affect the sensory quality of olives negatively (García et al., 1994). At a pH below 4.3, the sterilisation treatment would not be mandatory, and pasteurisation would be a more appropriate preservation method. Although acrylamide is not formed in the pasteurised product (Casado and Montaño, data not published), the sensory characteristics of this product are completely different to those of the sterilised product (Casado, Sánchez, Rejano, & Montaño, 2007).

3.2. Influence of additives on acrylamide formation in olive juice

The impact of different salts on acrylamide formation is shown in Fig. 2. $NaHSO_3$ is a preservative and antioxidant compound, which is frequently used in the vegetable processing industry. In Spain, the maximum amount permitted in brined vegetables is 100 mg/kg, expressed as SO_2, with the exception of table olives, in which this additive is not permitted (Ministerio de Sanidad y Consumo, 2002). The acrylamide reduction at 1.5 mM $NaHSO_3$, which corresponds to about 100 mg/l expressed as SO_2, was non-significant ($p < 0.05$), but significant reductions were obtained at higher concentrations (37% at 25 mM; 85% at 50 mM). $NaHSO_3$ has been found to reduce acrylamide formation in fried potatoes (Ou, Lin, Zhang, Huang, Sun, & Fu, 2008). Those authors suggested that $NaHSO_3$ may inhibit the production of intermediates that induce formation of acrylamide. In a cracker model based on wheat flour and water, $NaHSO_3$ was demonstrated to enhance acrylamide elimination (Levine & Smith, 2005).

NaCl is used in the table olive processing industry to impart the salty taste of the final product. Black ripe olives are usually packed with a cover brine containing 2–4% (w/v) NaCl (Sánchez, García, & Rejano, 2006). NaCl was tested at a concentration of 500 mM, which corresponds to 2.9% (w/v). A significant (30%) reduction of acrylamide was obtained. However, the possible use of this salt to control the formation of acrylamide in the actual product is limited because of its negative impact on olive taste at higher concentrations. Other sodium salts tested, such as NaH_2PO_4 and $Na_2B_4O_7$ at 100 mM level, also showed acrylamide-reducing effects, with reductions of 27% and 15%, respectively. Significant reductions of acrylamide with NaCl have also been found in potato crisps (Mestdagh et al., 2008) as well as in model solutions (Gökmen & Senyuva, 2007).

$CaCl_2$ is a firming agent, frequently added to the cover brine of packed table olives to improve olive firmness. At the 50 mM level (corresponding to 0.55%, w/v), this salt provoked a significant (24%) increase of acrylamide in olive juice (Fig. 2). This is in contrast to the acrylamide-reducing effect of $CaCl_2$ at a similar concentration in model systems or potato products (Gökmen & Senyuva,

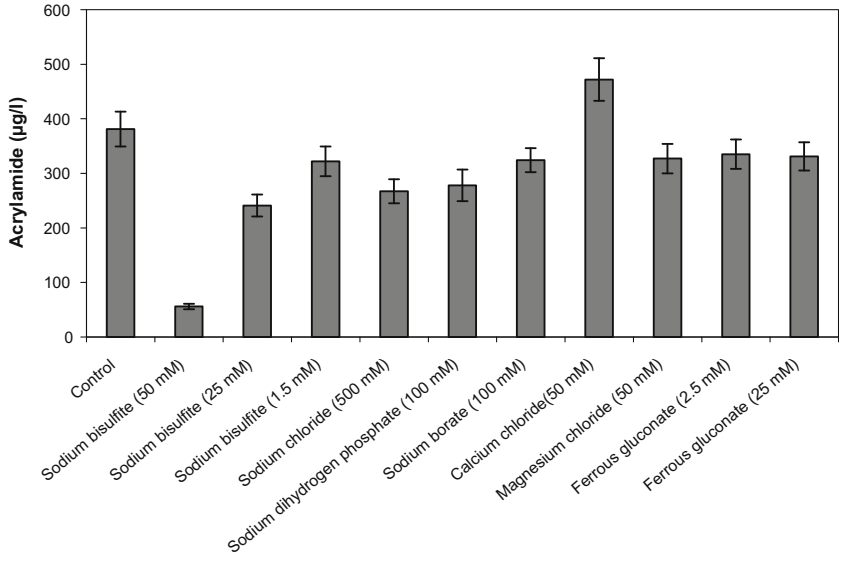

Fig. 2. Effect of different salts on the content of acrylamide in olive juice heated at 121 °C for 30 min. Data represent mean values ($n = 2$). Error bars indicate 95% confidence intervals, which were calculated taking into account that the repeatability (CV) of the heating procedure was 6%.

2007; Mestdagh et al., 2008; Ou et al., 2008), which has been attributed to the Ca^{2+} complexation with amines and some intermediates of the Maillard reaction products. This appears to confirm previous suggestions that acrylamide formation in olives follows a different pathway (Amrein et al., 2007; Casado & Montaño, 2008).

$MgCl_2$ has been demonstrated to exhibit a similar behaviour to that of $CaCl_2$ in inhibiting acrylamide formation (Mestdagh et al., 2008). At the 50 mM level, this salt had no significant acrylamide-reducing effect in olive juice (Fig. 2).

Ferrous gluconate is a permitted additive in black ripe olive processing. It is normally added at 10–40 ppm level, expressed as Fe, to prevent deterioration of the black colour in the packed product (Sánchez et al., 2006). At concentrations of 2.5 mM (corresponding to 150 ppm, expressed as Fe, which is the maximum permitted level in black ripe olives), and at even higher concentration (e.g. 25 mM), ferrous gluconate showed no significant effect on acrylamide concentration (Fig. 2).

The effect of different amino acids, added at the 50 mM level, on acrylamide formation is shown in Fig. 3. Cysteine showed the most-pronounced reducing effect, with a reduction of 50%. This result, which can be attributed to Michael-type reactions between the sulphydryl group of cysteine and acrylamide (Stadler et al., 2004), has also been observed in other model systems, although with different percentages of reduction: Mestdagh et al. (2008) found an acrylamide reduction of about 92% in a potato model solution. The amino acids Lys, Gly, and Arg showed similar acrylamide reductions, ranging between 24% and 27% (Fig. 3). Significant reductions due to addition of Lys or Gly have been found by other authors in both model solutions and actual food (Claeys et al., 2005; Mestdagh et al., 2008). However, we have not found any reference on the addition of Arg to reduce acrylamide. Ala and Val showed lower acrylamide reductions (19% and 16%, respectively). The other amino acids tested (Asp, Glu, Gln, Ser, and Tyr) did not have any significant effect.

When olive juice was fortified with 50 mM Asn, there was a dramatic increase in acrylamide concentration – more than 1200% compared with an unfortified control (data not shown). This result indicates that free Asn can form acrylamide in sterilised olives, as generally accepted for foods based on potatoes or cereals (Friedman, 2003). However, in contrast to these foods, unheated black ripe olives contain very little free asparagine (about 1 μmol/kg fresh weight), with the yield of acrylamide from asparagine exceeding 100% (Casado & Montaño, 2008). This result is in agreement with the yield found by Amrein et al. (2007) for a sample of natural black olives in brine, which was heated at 120 °C for 40 min in a GC oven. Therefore, another – unknown – mechanism of acrylamide formation must be acting in the case of sterilised table olives.

Finally, two phenolic compounds (HT and DHPG), both with high antioxidant activities and naturally present in olives (Rodríguez, Rodríguez, Fernández-Bolaños, Guillén, & Jiménez, 2007), were tested as additives. The two compounds were separately added at three different levels: 12, 25, and 50 mM, the first level being of the same order of magnitude as the concentration of HT in the juice of green table olives (Romero et al., 2004). The effect on acrylamide content in heated olive juice was not significant ($p < 0.05$) in any case (Fig. 4).

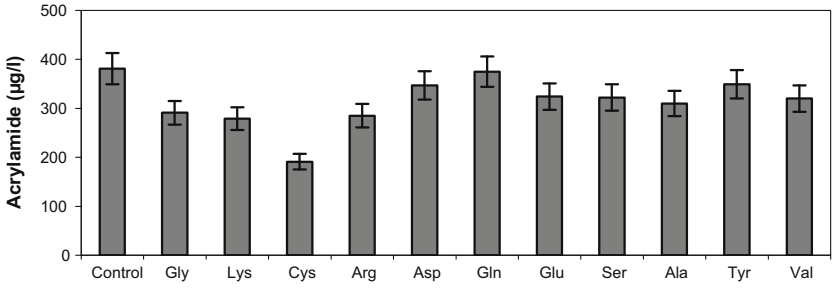

Fig. 3. Effect of amino acids on the content of acrylamide in olive juice heated at 121 °C for 30 min. All amino acids were L-isomers and were added at 50 mM level. Data represent mean values ($n = 2$). Error bars indicate 95% confidence intervals, which were calculated taking into account that the repeatability (CV) of the heating procedure was 6%.

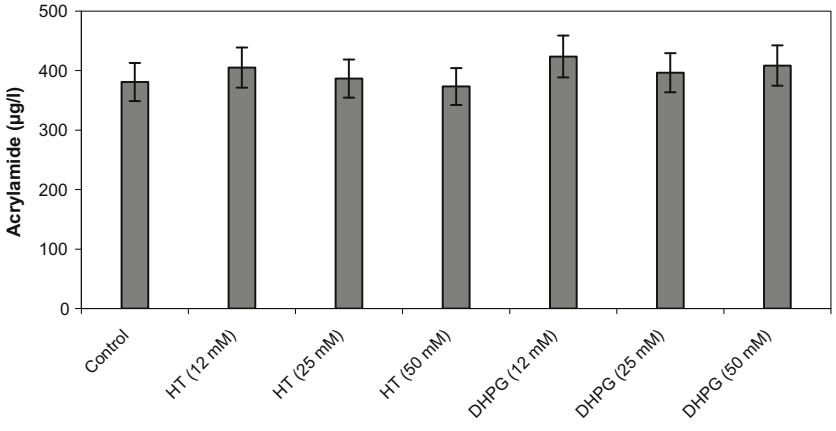

Fig. 4. Effect of two olive polyphenols, hydroxytyrosol (HT) and 3,4-dihydroxyphenyl glycol (DHPG), on the content of acrylamide in olive juice heated at 121 °C for 30 min. Data represent mean values ($n = 2$). Error bars indicate 95% confidence intervals, which were calculated taking into account that the repeatability (CV) of the heating procedure was 6%.

3.3. Influence of additives in black ripe olives

Based on the results obtained in the olive juice model system, $NaHSO_3$, Cys, and Arg were chosen to test their effects on acrylamide formation in the actual product (black ripe olives). Although $CaCl_2$ had no effect in reducing the acrylamide level in olive juice, this salt was also tested for comparison purposes. On the other hand, Lys – which showed a reducing effect similar to Arg – was ruled out because of its susceptibility to form cross-linked amino acids such as lysinoalanine in alkaline conditions (Friedman, 1999). In addition, other compounds and several natural products were tested in black ripe olives for the following reasons. Met was tested because it has been reported to decrease the acrylamide content in heated foods (Levine & Smith, 2005). NAC and GSH have been reported to have a behaviour similar to that of Cys in many respects (e.g. both SH-containing compounds are reducing agents that inhibit browning reactions in fruits and vegetables). Therefore, it can be postulated that they could be similarly effective in minimising the acrylamide content in ripe olives. Although the phenolic compounds HT and DHPG did not have any effect on the acrylamide level in olive juice (see previous section), we decided to test other antioxidants, such as propyl gallate, which is frequently used in the food industry to prevent rancidity in fatty foods, as well as different natural products (oregano, rosemary, tea, garlic) with well-documented antioxidant activities, attributed to their high content in phenolic compounds (Khan & Mukhtar, 2007; Miean & Mohamed, 2001; Zheng & Wang, 2001). Garlic is also rich in organosulphur compounds, especially S-substituted cysteine sulphoxides and γ-glutamyl peptides (Ichikawa, Ide, Yoshida, Yamaguchi, & Ono, 2006). Of the above natural products, rosemary has been found to reduce acrylamide content in bread (Hedegaard, Granby, Fradsen, Thygesen, & Skibsted, 2008).

$NaHSO_3$ at a concentration of 1.5 mM did not have any significant ($p < 0.05$) effect on acrylamide content in black ripe olives compared with the control, but at the 25 mM level provoked an acrylamide reduction of 100% (Table 1). This reduction is greater than that observed in olive juice (−37%, previous section). From a sensory standpoint, there were only slight differences with control in olive colour and flavour. Moreover, at this level, the panellists did not detect any abnormal or unpleasant taste.

$CaCl_2$ at 50 mM level did not show a significant ($p < 0.05$) effect in reducing the acrylamide content. Therefore, the increase found in the olive juice model system was not observed when this additive was added to ripe olives.

Similarly to the tendency observed for $NaHSO_3$ and $CaCl_2$, the reducing effect of Cys and Arg was higher in the actual product than in the model system. Thus, acrylamide reductions of 100% and 43%, respectively, were obtained for these amino acids at the 50 mM level (Table 1). Unfortunately, Cys negatively affected the sensory quality of ripe olives: they showed a significant loss of black colour in comparison with control, and an unpleasant odour was clearly detected. Unpleasant off-flavours due to addition of Cys have also been found in other heated foods (Mestdagh et al., 2008; Ou et al., 2008). On the other hand, the addition of Arg did not significantly affect the olive colour, and its impact on olive flavour was not negative, although the panellists found significant differences with the control. Olive firmness was slightly affected by addition of this amino acid.

The addition of NAC (50 mM) or GSH (25 mM) had the same effect as that of Cys on acrylamide content. Similarly to Cys, both compounds negatively affected the sensory characteristics. Thus, the same unpleasant odour that was found with added Cys was also detected in both cases, although with lower intensity. This drawback appears to be a general rule when any compound with an SH group is added to heated foods (Claeys et al., 2005). In contrast, the addition of Met (i.e. a sulphur amino acid without an SH group) had no negative impact on sensory quality of the product, but neither did it show any acrylamide-reducing effect.

Propyl gallate had no significant ($p < 0.05$) effect on acrylamide level or on sensory characteristics of olives. The concentration tested for this antioxidant (0.9 mM) corresponded to its maximum permitted level in fatty foods (Ministerio de Sanidad y Consumo, 2002). Although a higher concentration was not tested, the results obtained in olive juice with other antioxidant compounds, such as

Table 1
Impact of various additives on the acrylamide content as well as on the physicochemical and sensory characteristics of black ripe olives (sterilised at 121 °C for 15 min).

Additive[a]	Acrylamide content[b]	pH	Titratable acidity (% lactic acid)	Combined acidity (*N*)	Salt (% NaCl)	Firmness difference with respect to control (Δ*F*)	Colour difference with respect to control (ΔR_{700})	Panel test[c]
Control	273 ± 28	6.15	0.05	0.032	2.22	–[d]	–[e]	
$NaHSO_3$ (1.5)	244 ± 10	6.32	0.04	0.028	2.12	−0.9	+0.2	
$NaHSO_3$ (25)	ND*	5.93	0.18	0.036	2.37	+0.4	+1.1*	*
$CaCl_2$ (50)	233 ± 4	6.02	0.04	0.027	2.61	+10.8*	+0.1	
Cys (50)	ND*	6.04	0.23	0.033	2.43	−0.1	+2.0*	*
NAC (50)	ND*	6.16	0.05	0.059	2.37	−5.3 *	+0.5*	*
GSH (25)	ND*	6.06	0.10	0.056	2.05	−1.9	+0.5*	*
Met (50)	261 ± 3	6.08	0.08	0.044	2.69	+1.3	+0.1	
Arg (50)	156 ± 9*	6.20	0.08	0.037	2.03	−3.9 *	+0.3	*
Propyl gallate (0.9)	280 ± 5	6.36	0.04	0.028	2.14	+1.2	-0.3	
Green tea (3)	275 ± 11	6.13	0.05	0.031	2.15	+5.1*	+0.2	
Rosemary (3)	265 ± 3	5.94	0.05	0.029	2.05	+0.8	−0.4*	
Orégano (3)	264 ± 2	5.57	0.06	0.032	2.12	+2.4	−0.1	
Raw garlic (15)	303 ± 19	6.07	0.05	0.033	2.04	+0.2	+0.2	
Blanched garlic (15)	209 ± 2*	6.09	0.04	0.031	2.00	+0.1	+0.2	*
Blanched garlic (80)	251 ± 2	6.03	0.10	0.045	2.01	-1.6	0.0	

*Significant difference with respect to control ($p < 0.05$).
[a] Concentration at equilibrium, in brackets, for compounds are in mM, while natural products are in g/kg net weight.
[b] Mean ± standard error, values in μg/kg pulp.
[c] Paired comparison tests were done only for samples with significant reduction in acrylamide content compared with control.
[d] In control, absolute firmness of olives was 19.6 N/g.
[e] In control, surface colour of olives was $R_{700} = 3.53$.

HT or DHPG, suggest that polyphenols are not effective acrylamide-reducing agents in the case of ripe olives.

Of the natural products tested, only blanched garlic showed a significant reduction of acrylamide content (Table 1). In this case, olive firmness and colour were not significantly affected, but a few panellists noted a distinct "garlic flavour". The acrylamide-reducing effect can be attributed to the presence of S-alk(en)yl-L-cysteine sulphoxides, mainly S-allyl-L-cysteine sulphoxide (alliin). Blanching treatment results in the deactivation of the enzyme alliinase, which transforms the S-alk(en)yl-L-cysteine sulphoxides into thiosulphinates (the major one being diallylthiosulphinate, or allicin) when raw garlic is minced (Rejano, De Castro, Sánchez, Casado, & Montaño, 2004). Assuming an alliin content in garlic of 1 g/100 g fresh weight (Iberl, Winkler, Müller, & Knobloch, 1990), the addition of blanched garlic at 15 g/kg net weight would correspond to an alliin concentration of 0.9 mM. A higher dosage of blanched garlic (e.g. 80 g/kg net weight) did not show any significant acrylamide-reducing effect. Apparently, the reducing effect is counteracted by a higher amount of acrylamide precursors, such as Asn, from garlic. It has been reported that Asn is a major free amino acid in blanched garlic, with a level similar to that found in raw potatoes (Lee & Harnly, 2005). We did not test the addition of pure alliin, because this compound is expensive, making its possible application in ripe olive processing unacceptable to the industry.

In summary, of the additives tested in the present work, $NaHSO_3$ at 25 mM had the highest impact on acrylamide level in black ripe olives, without a negative repercussion on sensory quality. Although this compound is currently not permitted as an additive in table olives, we believe that – taking into account the above result – its use in the case of ripe olives should be reconsidered. Although optimisation experiments were not carried out in the present work, the above concentration (corresponding to $\approx$1600 mg/kg expressed as SO_2) is not excessive. In fact, it is lower than the maximum level permitted in dried vegetables such as apricots, peaches, grapes, prunes, and figs (2000 mg/kg expressed as SO_2; Ministerio de Sanidad y Consumo, 2002). Arg can also be proposed as another compound of interest for reducing acrylamide in ripe olives with a limited impact on sensory quality. This amino acid is considered a semi-essential amino acid because even though the body normally makes enough of it, supplementation is sometimes needed. In addition, Arg has been reported to have many health benefits (Wu, Meininger, Knabe, Bazer, & Rhoads, 2000), so that its use might be readily taken up by the olive industry.

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