**EFFECTS OF SELECTED AMINO ACIDS AND WATER-SOLUBLE
VITAMINS ON ACRYLAMIDE FORMATION IN A RIPE OLIVE MODEL
SYSTEM**

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Abstract

A ripe olive model system was used to evaluate the potential inhibiting effects on acrylamide formation from a set of amino acids and water-soluble vitamins. The system was based on ripe olive juice heated at 121 °C for 30 min in a stainless steel tubular reactor. The most potent acrylamide inhibitors were proline and sarcosine, both with inhibition rates of ~75% at a 100 mM level. In addition, glycine, ornithine, taurine, and γ -amino butyric acid were effective (50-65% inhibition) while the rest of the compounds demonstrated weak or non-significant effects. Acrylamide contents in the model system were found to be highly correlated with the corresponding contents in the real product. The kinetic pattern for the formation of acrylamide in the absence and presence of two selected amino acids, added separately or together, was well fitted using a simple logistic function.

Keywords: Acrylamide, Amino acids, Food model system, Kinetics, Mitigation, Vitamins

1. Introduction

Acrylamide (AA) has been a focus of attention by the scientific community in recent years. This compound has been classified by the International Agency for Research on Cancer (IARC) as a probable human carcinogen (IARC, 1994). In early 2002, high AA levels were reported in potato products and cereals, such as fried potatoes, baked potatoes, bread, breakfast cereals, and biscuits (Tareke et al., 2002). Subsequent studies confirmed this finding (FDA, 2006) and also reported high AA levels in other products such as ripe olives (Casado and Montaña, 2008). Previous surveys also showed that AA was not detected in the olives of other processing types (e.g. Spanish-style green olives, directly brined olives, etc.), or in other low acid canned vegetables such as asparagus, green beans, and spinach (FDA, 2006).

Ripe olives (also called “Californian-style table olives”) are one of the most important classes of table olive commercialized in the world. In this type of processing the olives are treated with a series of dilute NaOH solutions (lye) to remove their natural bitterness, which is caused by glucoside oleuropein. Between lye treatments, the fruits are aerated. During this operation, the fruits are progressively darkened due to polyphenol oxidation. After the lye treatments and oxidation, the olives are washed several times with water to remove most of the residual lye, reaching a final pH of around 7, and placed in 3-5% brine with ferrous gluconate or ferrous lactate to maintain their color (Sánchez et al., 2006). Finally, the olives are canned in mild salt brine, and heat sterilized (generally at 121-126 °C).

The use of additives for the potential inhibition of AA formation is a strategy that has been investigated in different foods, including ripe olives. Of the additives tested in this product, only sodium bisulfite was able to totally eliminate AA with a

negligible repercussion on sensory quality (Casado et al., 2010). However, sodium bisulfite is currently not permitted as an additive in table olives in accordance with European regulation (Commission Regulation (EU) No 1129/2011). Therefore, studies to find other additives to be used by the table olive industry with significant AA-reducing effects and without any negative effects are necessary. Amino acids or vitamins are especially attractive because their addition to food may also improve its nutritional value. However, the results previously found with a few amino acids in ripe olives were generally not satisfactory (Casado et al., 2010). Cysteine at 50 mM was demonstrated to be a strong inhibitor of AA formation in ripe olives, but did generate unpleasant off-flavors. In contrast, arginine and methionine at 50 mM had no negative impact on the sensory quality of ripe olives, but their AA-reducing effects were little or negligible. Protein amino acids such as tryptophan, proline, and histidine have demonstrated significant AA-reducing effects in asparagine-glucose model systems (Koutsidis et al., 2009). It has been suggested that these amino acids could form amino acid-AA adducts through Michael addition type reactions thereby reducing the AA content (Friedman and Levin, 2008; Koutsidis et al., 2009). Taurine, a non-protein amino acid, has been reported to be an inhibitor of AA formation in aqueous and potato chip model systems (Shin et al., 2010). Several water-soluble vitamins were reported to significantly inhibit the formation of AA in model systems and in fried potatoes (Zeng et al., 2009; Yuan et al., 2011). Although the action mechanism has not been characterized, the presence of nucleophilic groups, in particular, an amino group in some vitamins, might contribute to their inhibitory activity against AA formation.

The reduction of AA levels by using additives may significantly affect the kinetic behavior of acrylamide formation. The kinetics of AA formation have been previously studied in model systems of asparagine and glucose (Claeys et al., 2005;

Knol et al., 2005; Zhang and Zhang, 2008), potato chips (Granda and Moreira, 2005), potato crisps (Knol et al., 2009), and potato powder (Franke et al., 2009). Empirical models have been proposed for modeling the formation of AA in foods and model systems (Corradini and Peleg, 2006). In fact, the “Logistic-Fermi” and “Logistic-Exponential” models have been used to fit the formation of AA in fried potato crisps (Knol et al., 2009) and in an asparagine-glucose model system (Zhang and Zhang, 2008). Using these models that only give a mathematical description of the formation or degradation of AA in food bypasses the problem of considering all the mechanisms that occur during the processing of foods. Besides, AA precursors in olives are still unknown. In the case of ripe olives the AA precursors appear to be different from those in other heated foods. Thus, it is well-demonstrated that the Maillard reaction from amino acids, mainly asparagine, along with reducing sugars, represents the main formation route of AA in potatoes products (Amrein et al., 2004; Taubert et al., 2004), roasted almonds (Amrein et al., 2005) and roasted tea (Mizukami et al., 2006). However, previous studies in olives showed no correlation between their AA content and any of the sugars or amino acids determined before sterilization, which appears to indicate that these compounds are irrelevant as AA precursors in olives (Amrein et al., 2007; Casado and Montaña, 2008). The aim of the present work was twofold. The first aim was to assess the efficiency of selected amino acids and water-soluble vitamins to eliminate or reduce AA in a ripe olive model system, which mimicked the chemical composition and the heat treatment of ripe olives. For comparison purposes, a strong inhibitor of AA formation in ripe olives, namely sodium sulfite, was also tested. To evaluate the reliability of the model system, some results obtained from the model system were compared with those obtained with the real product. The second aim was

to investigate the kinetic profile of AA formation in the ripe olive model both in the absence and presence of selected additives.

2. Materials and methods

2.1. Chemicals

Individual amino acids (L-arginine, glycine, L-tryptophan, L-proline, L-histidine, DL-ornithine hydrochloride, taurine, sarcosine, and γ -amino-*n*-butyric acid), vitamins (thiamine hydrochloride, VB1; nicotinic acid, VB3; pyridoxine, VB6; biotin, VB7; and sodium- L -ascorbate, VC), and sodium sulfite were supplied by Sigma-Aldrich (St. Louis, MO). Alliin (*S*-allyl-L-cysteine sulfoxide) was isolated from garlic powder by the method of Mochizuki et al. (1997). The garlic powder was prepared as follows: fresh garlic cloves were frozen in liquid nitrogen, immediately peeled, and lyophilized. Methiin (*S*-methyl-L-cysteine sulfoxide) was synthesized as described by Shen and Parkin (2000) using S-methyl-L-cysteine (Sigma) as the starting material. Deionized water (Milli-Q; Millipore Corp.) was used throughout. All reagents and chemicals used for the AA analysis were as described by Casado and Montaña (2008). All other chemical and solvents were of analytical grade from various suppliers.

2.2. Preparation of ripe olive model systems

Green olives (Hojiblanca cultivar) were stored in 2.4% acetic acid for about three months before processing, and were then subjected to darkening as follows: olives (≈ 22 kg) were treated in a horizontal stainless steel cylindrical container (0.4 m

diameter x 0.7 m length) with a lye solution of 3%, which progressively penetrated the flesh until the alkali reached the pit. Next, the lye was removed and the olives were washed with water until the pH reached 8.0. During lye treatment and washing, air was injected through the bottom of the container. Pressurized air is introduced through 15 spigots (0.5 mm diameter) uniformly located in the bottom of the container so that the oxidation process is uniform. Then, a 0.1% ferrous gluconate solution with pH corrected to 4.5 was added to fix the black color (López-López et al., 2009). One portion of ripe olives (≈ 10 kg) was then subjected to the following operations: pitting, homogenization using a mixer, filtration through cheesecloth, and centrifugation at 20,000g for 20 min. After separation of the oil, the resulting juice was stored at -30 °C until the moment of performing the different tests. The selected compounds were added separately to juice in known concentrations. The addition levels of the amino acids were 50, 100, and 200 mM whereas vitamins were assayed at 25, 50, and 100 mM. In all cases, prior to heating, the pH of the mixture was adjusted to 7. Heat treatment was performed by placing olive juice (1 mL) in a custom-made cylindrical stainless steel tubular reactor (internal diameter 0.7 cm, length 3.0 cm) having one end closed. The reactor was sealed with a stainless steel tube plug, and then heated in an oil bath at 121 ± 1 °C for 30 min. The bath was equipped with a stirrer to ensure a homogeneous temperature in the oil. After heating, the sample was immediately cooled in ice water for 3 min to stop any further reaction and analyzed for its AA content. The temperature profile inside the reactor tube was obtained using a stainless steel temperature probe (Pt100 sensor) coupled to a Crison thermometer model 620/3 (Crison Instruments, Barcelona, Spain). The probe (3 mm diameter) was inserted through the hole of a rubber washer (14 mm external diameter, 1 mm internal diameter, 3 mm thickness) placed under a nut previously connected to the open end of the reactor. The temperature profile is shown in

Figure 1. The control was treated with the same experimental steps but without additives. All heating experiments were performed in triplicate.

2.3. Preparation of packed ripe olives

Another portion of ripe olives was packed in “A314” glass bottles (145 g of pitted olives with 170 mL of brine capacity) and covered with brine containing 3% NaCl, 0.015% ferrous gluconate, and the corresponding compound (Na_2SO_3 , Pro, Sar, or Gly). Compounds were added to give fixed equilibrium values of 100 and 200 mM, except for Na_2SO_3 , which was added at 10 and 20 mM. A control product was prepared using the same brine without additives. If necessary, before the olives were covered, the pH of the packing brine was adjusted to 6.5-7.0 by adding NaOH or HCl. Before sealing, the bottles were filled with hot brine (70 °C). Two bottles from each treatment were heated at 121 °C for 15 min in a computer-controlled retort (Steriflow, SAS, Paris, France). Before starting the retort cycle, the bottles were pre-heated at 50 °C for 10 min. In the heating phase or come-up time of the retort cycle, the water process was heated by steam in the primary circuit of heat exchanger; in the holding phase, temperature (121 °C) and pressure (2.8 bar) were stabilized; and finally, in the cooling phase, cold water was injected in the heat exchanger in order to cool down the process water. The cycle period was 55 min. Sterilization treatment was performed in triplicate. After 3 months storage, AA analysis was carried out.

2.4. Kinetic studies

Ripe olive juice without (control) and with selected additives at fixed concentrations were heated at 121 °C in tubular reactors for selected heating times (5,

15, 25, 35, 45, 55, and 65 min). Although heating times longer than 30 min are not of practical significance in industrial ripe olive processing, prolonged heating times were included in order to consider a possible degradation step of AA. Each heat treatment was performed at least in duplicate. The obtained data were analyzed using different kinetic models. The Logistic-Fermi model (Eq. 1) proposed by Corradini and Peleg (2006) describes the formation of AA by a logistic function and the degradation by a Fermi-type function:

$$C(t) = \left[\frac{a(T)}{1+\exp\{k_1(T)[t_{c1}(T)-t]\}} - \frac{a(T)}{1+\exp[k_1(T)t_{c1}(T)]} \right] \cdot \frac{1}{1+\exp\{k_2(T)[t-t_{c2}(T)]\}} \quad (1)$$

where $C(t)$ is the concentration of AA, $t_{c1}(T)$ and $t_{c2}(T)$ are temperature-dependent time characteristics for the inflection points in the formation (t_{c1}) and degradation (t_{c2}) of AA, $k_1(T)$ and $k_2(T)$ are temperature-dependent steepness parameters around the inflection points for the formation (k_1) and degradation (k_2) of AA and $a(T)$ serves as a temperature-dependent “scale factor” for the AA concentration.

The Logistic-Exponential model (Eq. 2) differs from the above model in the function that describes the degradation of AA:

$$C(t) = \left[\frac{a(T)}{1+\exp\{k_1(T)[t_{c1}(T)-t]\}} - \frac{a(T)}{1+\exp[k_1(T)t_{c1}(T)]} \right] \cdot \exp\left(-\frac{t}{\tau(T)}\right) \quad (2)$$

where $\tau(T)$ is a temperature-dependent characteristic of time.

Since AA was apparently not degraded as a result of the applied heat treatments the simple logistic function (Eq. 3) describing the AA formation was also studied:

$$C(t) = \frac{a(T)}{1+\exp\{k_1(T)[t_{c1}(T)-t]\}} \quad (3)$$

Since the R^2 value cannot be used to evaluate nonlinear models, the quality of fit was expressed as the pseudo- R^2 value:

$$\text{pseudo-}R^2 = 1 - (\text{SS}_{\text{residual}}/\text{SS}_{\text{corrected total}}) \quad (4)$$

where $\text{SS}_{\text{residual}}$ stands for the sum of squares of residuals and $\text{SS}_{\text{corrected total}}$ for the corrected total sum of squares, that is, the squared difference of the observed value from the mean summed over all observations (Motulsky and Christopoulos, 2003).

The adequacy of the kinetic model was also evaluated graphically by plotting the predicted values against the experimental values.

It is known that that the coordinates of the inflexion point (P) of the above logistic function are $(t_{c1}, a/2)$ and the curve's slope (formation rate) at this point satisfies the equation:

$$dP/dt = k_1P(1 - P/a) \quad (5)$$

Therefore,

$$dP/dt = k_1a/4 \quad (6)$$

2.5. Analysis of AA

Determination of AA in olive juice or ripe olives was carried out as described previously (Casado and Montaña, 2008). AA was determined by gas chromatography-mass spectrometry (GC-MS) after bromination and ethyl acetate extraction of the 2,3-dibromopropionamide using $^{13}\text{C}_3$ -AA as an internal standard.

2.6. Statistical analysis

All experiments were performed in triplicate and statistically analyzed by means of analysis of variance (ANOVA) on a significance level of $p=0.05$. The software Statistica version 7.0 (Statsoft Inc., Tulsa, OK) was used.

3. Results and discussion

3.1. Effect of additives on AA formation in ripe olive model system

In control juice, 319 ± 50 $\mu\text{g/L}$ (mean $\pm$ SD, $n=3$) of AA was formed after 30 min heating at 121 °C. As expected, sodium sulfite was found to be a very effective AA-reducing compound. The reduction rate reached 91% after adding 10 mM of sodium bisulfite (data not shown). After the addition of 20 mM or more, the AA formation was completely inhibited, which confirmed the effectiveness of bisulfite as a strong inhibitor of AA formation in ripe olives as previously reported by Casado et al. (2010). Of the tested protein amino acids (Figure 2), proline was the most potent inhibitor of AA formation (reduction rates of 61%, 78%, and 91% at 50 mM, 100 mM, and 200 mM, respectively) followed by glycine (30%, 54%, and 64%). Tryptophan inhibited AA formation (~36% reduction), but reduction was not dose-dependent. Arginine reduced the formation of AA at concentrations of 100 and 200 mM (reductions of 24% and 42%, respectively), but it was not effective at the 50 mM level. Finally, histidine was only effective at the highest concentration assayed (39% reduction at 200 mM). It has been demonstrated that the above-mentioned amino acids can form amino acid-AA adducts

through Michael addition type reactions thereby reducing the AA content, although a clear identification in the specific condensation product was not obtained in the case of arginine (Koutsidis et al., 2009; Adams et al., 2010).

Of the non-protein amino acids, sarcosine (*N*-methyl glycine) showed the highest inhibition rates, similar to those of proline (Figure 3). The higher reactivity of sarcosine in comparison with the corresponding primary amine containing amino acid (i.e. glycine) is noteworthy. Ornithine, taurine, and γ -amino-*n*- butyric acid also gave good inhibition rates (50-65% at 100 mM). Shin et al. (2010) reported significant reductions of AA formation in a fried potato chip model when, prior to frying at 170 °C for 3 min, the potato slices were soaked in 0.1-2% taurine solution for 30 min. However, to our best of knowledge, the potential use of other nonprotein amino acids such as sarcosine, ornithine and γ -amino-*n*- butyric acid to decrease AA formation in processed foods has not been previously suggested. In addition, all these nonprotein amino acids could be particularly interesting from a healthy standpoint, as they are widely used as components in nutritional supplements.

Natural compounds are attractive candidates to be added as inhibitors of AA formation in processed foods. In the present study, the non-protein sulfur-containing amino acids alliin and methiin were tested at concentrations between 25-100 mM (Figure 3). These two *S*-alk(en)yl-L-cysteine sulfoxides are present in relatively high concentrations in *Allium* vegetables (Horie and Yamashita, 2006) and they are thought to be beneficial to health. Unfortunately, alliin did not show a significant AA-reducing effect compared to the control juice in any case while methiin only reduced AA formation at high concentrations with reduction rates rather modest(<40%). Moreover, as a serious drawback, a distinct “garlic odor” after the heat treatment was noted in all samples with added alliin or methiin. This odor can be attributed to thermal breakdown

products of these compounds (Kubec et al., 1997; Kubec et al., 1998). In our previous study (Casado et al., 2010), the addition of minced blanched garlic at 15 g/kg of ripe olives was reported to reduce the AA content in ripe olives by 23%, which was hypothesized to be due to the presence of the cysteine sulfoxides in garlic, mainly alliin. The above-mentioned amount of blanched garlic would correspond to alliin and methiin concentrations of ~1 mM and ~0.1 mM, respectively, assuming that the levels of these compounds in garlic are 35 and 3.5 mg/g of dm, respectively, and there are no losses during the blanching treatment (Montaño et al. 2011; Beato et al., 2012). These concentrations are 1-3 orders of magnitude lower than the concentrations tested in the present study. Therefore, the above results appear not to confirm the hypothesis that cysteine sulfoxides are involved in the AA reducing effect of blanched garlic, suggesting that other unknown compounds would be responsible for this effect. It has been reported that allicin (diallylthiosulfinate), which is formed from alliin by the action of the enzyme alliinase when raw garlic is minced, effectively reduces AA formation (>50% reduction) in an asparagine/fructose model system containing 0.0375% allicin (Yuan et al., 2011). However, allicin is expected not to be present in blanched garlic, as blanching treatment results in the deactivation of alliinase (Rejano et al., 2004).

Of the tested water-soluble vitamins, only VB1 significantly reduced the AA content compared to the control juice (Figure 4). However, the addition of this vitamin imparted a noticeable bad odor to heated juice. Presumably, this could be related to the presence of a thiazole ring as a component of the VB1 molecule. The other water-soluble vitamins had no significant AA-reducing effect in the olive model system in general, which is in contrast with the significant AA reductions reported in other model systems (Zeng et al., 2009; Yuan et al., 2011).

3.2. Comparison between the olive model system and the real product

Results obtained for a few additives in the model system were compared with those obtained for the same additives in the real product (packed ripe olives). AA formed in the latter product without additives was 598 µg/kg in olive pulp. A good correlation ($R^2 = 0.941$) between the AA contents obtained in the model system and those obtained in the real product was found (Figure 5), with a slope $\pm$ SE of 1.72 ± 0.14 . The reason for the higher AA contents in packed olives compared to model system could be related to the amount of AA precursors in each case. The model system based on olive juice is a simplified representation of the real product (packed olives). Recently, we have demonstrated that peptides and/or proteins are precursors of AA formation in sterilized olives (Casado et al., 2013). These compounds are not only in the olive juice (which more properly should be called olive water, i.e. the aqueous fraction of olive pulp) but also in the solid fraction linked to olive components (eg., fiber) and even in the olive oil (Hidalgo et al., 2001). Therefore, the amount of AA precursors in packed olives should be higher than in olive juice, which would explain the higher AA contents in packed olives.

The above correlation between the model system and the real product indicates that one could use the ripe olive model system as a simple and reliable tool for screening compounds with potential inhibitor effects on the AA formation in ripe olive processing. However, the obtained linear function could not be applicable under different conditions with respect to those used in the present study (i.e. olive juice and real product made with Hojiblanca olives processed by using the same darkening method). Changes in the darkening method or olive cultivar could affect the kinetic behavior of AA formation, which in turn would affect the final AA level in ripe olives (Casado and Montaña, 2008).

3.3. Kinetic studies

The kinetic profile of AA formation in the ripe olive model at 121 °C for 5-65 min in the absence (control) and presence of selected additives is shown in Figure 6. The additives selected were sarcosine at 100 mM and arginine at 200 mM, which are examples of amino acids with strong and weak capacities, respectively, to inhibit AA formation. After a “lag-phase” of at least 5 min, in which AA formation was negligible, AA content increased and eventually reached an equilibrium value. This “lag-phase” was expected taking into account that AA formation in foods appears to begin at temperatures around 120 °C (Stadler et al., 2002) and this temperature inside the tubular reactor was just reached after 10 min heating (Figure 1). When the “Logistic-Fermi” and “Logistic-Exponential” models were applied, the fit of these empirical models to our data did not give satisfactory results in any case (data not shown), which was probably due to the absence of a degradation step of AA and the relatively low number of data points. However, when a simple logistic pattern based on only three parameters was used (Eq. 3), the fit was quite good for the data set measured (Figure 6). Calculation results of the kinetic parameters describing the formation of AA in each case along with the corresponding pseudo- R^2 values are shown in Table 1. The goodness of fit of the model on the data was confirmed graphically by the scatter plots shown in Figure 7, where a high correlation was found between predicted and experimental values in all cases. The parameters k_1 and t_{c1} in the juices with additives were not significantly different compared to the control. In other words, the addition of the amino acids to the model system did not appear to influence the nature of the kinetic model. Nevertheless, the parameter a was significantly lower in the additive-containing samples compared to the control. A consistently lower a value indicates a lower level of AA production. This

means that sarcosine and arginine at the concentrations tested had a significant antagonist effect on the AA formation at 121 °C. When a mixture of 100 mM Sar + 200 mM Arg was tested, the calculated parameter a was significantly lower than those obtained for the amino acids separately, but again k_1 and t_{c1} did not significantly change. This result demonstrates that the AA reducing effect of the above mixture was significantly higher than the two amino acids added separately. It must be stressed that the parameter k_1 does not actually represent a formation rate, but this can be calculated from the curve's slope at the inflexion point according to equation 6. The obtained values of the formation rate (Table 1) were consistent with the differences observed in the AA formation curves (Figure 6) where control system showed a much faster formation compared to the systems with AA inhibitors.

The logistic model (or empirical models in general) as mentioned above only describe the AA formation kinetics but they do not give much insight into the mechanism behind the AA formation. A logistic function was previously used by Granda and Moreira (2005) to model the kinetics of AA formation in traditional fried potato chips. Knol et al. (2009) used the “Logistic- Exponential” model to make predictions for the AA formation in potato crisps based on the strong correlation between the parameter a obtained by the model and the reducing sugar content. Since AA precursors in olives are still unknown, a similar correlation between the parameter a derived from the logistic function and the content of AA precursor cannot be determined as yet. Studies are in progress which use the tubular reactor methodology described in this work to identify the main AA precursors in olives. Once these studies are carried out and assuming that a good correlation between parameter a and precursor content is found, the next step would be to investigate the kinetics of AA formation in real food (i.e. ripe olives), under well-controlled conditions, and to apply the logistic

function to kinetic data. Models of the proposed kind might be used to simulate and predict the generation of AA in ripe olives. In addition, the obtained information might be useful for developing new strategies for the reduction or elimination of AA by decreasing or eliminating precursors.

4. Conclusion

The present study shows that our olive model system is a simple and reliable tool for the screening of additives as potential AA inhibitors in ripe olive processing. Excluding sulfite, the compounds which had the highest impact on the AA level were proline and sarcosine. Further research is currently underway to determine whether these compounds would be good candidates to decrease AA formation in ripe olive processing taking into account their impact on the sensory characteristics of the product. The ripe olive model system was useful in identifying the kinetics of AA formation at 121 °C. This was modeled using a simple logistic model, characterized by an increase in the AA content with heating time until an equilibrium value is eventually reached. Hopefully, this type of model could be used in the future to predict the formation of AA and to develop new strategies for its reduction or elimination in ripe olives.

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526

Figure captions

Figure 1. Temperature-time profile of samples heated in a custom-made cylindrical stainless steel tubular reactor (internal diameter 0.7 cm, length 3.0 cm).

Figure 2. Dose-response effect of selected protein amino acids on the acrylamide inhibition (percentage from control) in a ripe olive model system. Data values are mean $\pm$ SD (n=3). Bars with an asterisk indicate significant difference from the control (p<0.05). Abbreviations: Pro = proline, Gly = glycine, Trp = tryptophan, Arg = arginine, and His = histidine.

Figure 3. Dose-response effect of selected non-protein amino acids on the acrylamide inhibition (percentage from control) in a ripe olive model system. Data values are mean $\pm$ SD (n=3). Bars with an asterisk indicate significant difference from the control (p<0.05). Abbreviations: Sar = sarcosine, Orn = ornithine, Tau = taurine, and GABA = γ -aminobutyric acid.

Figure 4. Dose-response effect of selected water-soluble vitamins on the acrylamide inhibition (percentage from control) in a ripe olive model system. Data values are mean $\pm$ SD (n=3). Bars with an asterisk indicate significant difference from the control (p<0.05). Abbreviations: VB1 = thiamine hydrochloride, VB3 = nicotinic acid, VB6 = pyridoxine, VB7 = biotin, and VC = sodium- L -ascorbate.

Figure 5. Relationship between the acrylamide contents in ripe olive model system and those in real product (ripe olives).

Figure 6. Formation of acrylamide as function of heating time at 121 °C in ripe olive model system in the absence (control) and presence of two different acrylamide

550 inhibitors. The lines represent acrylamide predicted by the kinetic model (logistic
551 function with three parameters).

552 Figure 7. Plots of experimentally determined acrylamide levels and acrylamide levels
553 predicted by the kinetic model (logistic function with three parameters) in ripe olive
554 model system in the absence (control) and presence of two different acrylamide
555 inhibitors.

Table 1. Effect of additives on the kinetic parameters describing the formation of acrylamide fitted by logistic function (Eq. 3 in text)

	Logistic model ^a				
Sample	a ($\mu\text{g/L}$)	k_1 (min^{-1})	t_{c1} (min)	Rate ^b	pseudo- R^2
Control	$624 \pm 43\text{a}$	$0.09 \pm 0.02\text{a}$	$29 \pm 3\text{a}$	13.55	0.9590
+100 mM Sar (1)	$196 \pm 8\text{c}$	$0.12 \pm 0.02\text{a}$	$22 \pm 2\text{a}$	6.14	0.9714
+200 mM Arg (2)	$257 \pm 13\text{b}$	$0.14 \pm 0.03\text{a}$	$26 \pm 2\text{a}$	8.75	0.9454
(1) + (2)	$134 \pm 5\text{d}$	$0.20 \pm 0.05\text{a}$	$20 \pm 1\text{a}$	6.68	0.9341
^a Parameters are expressed as mean $\pm$ SE. Values of the same parameter with different letters are significantly different based on 95% confidence intervals. ^b Curve's slope at the inflexion point (equation 6 in text).					

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6

Figure 7