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Effect of Processing and Cooking Conditions on Onion (*Allium cepa* L.) Induced Antiplatelet Activity and Thiosulfinate Content

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ABSTRACT: *Allium* vegetables serve as sources of antiplatelet agents that may contribute to the prevention of cardiovascular disease. However, onion and garlic, the major *Allium* species, are usually cooked before consumption. Here, we examined the effect of cooking on onion *in vitro* antiplatelet activity (IVAA). Two different cooking systems (convection oven and microwaves) and several time−temperature variables were tested on whole bulbs, quarters of bulbs, and completely crushed bulbs, monitoring the degradation of sulfur antiplatelet compounds (e.g., thiosulfinates) by analysis of pyruvate levels. Although heating was, in general, detrimental for onion IVAA, the extent of this effect varied greatly, from unaffected antiplatelet activity (AA) (i.e., similar to raw onion) to a complete lost of activity, depending upon the manner in which onions were prepared prior to heating, the cooking method, and the intensity of the heat treatment. "Whole", "quarters", and "crushed" onions lost their IVAA after 30, 20, and 10 min of oven heating, respectively. The longer retainment of AA in intact bulbs was attributed to a later alliinase inactivation. Proaggregatory effects observed in samples subjected to the most intense oven and microwave heat treatments suggest that extensively cooked onions may stimulate rather than inhibit platelet aggregation. The efficacy of *Allium* species as antiplatelet agents, as affected by preparation and cooking conditions, is discussed.

KEYWORDS: *Allium cepa, antiplatelet activity, pyruvate, thiosulfinates, cooking*

INTRODUCTION

Allium vegetables, such as onion (*Allium cepa*) and garlic (*Allium sativum*), are recognized as antiplatelet agents that may contribute to the prevention of cardiovascular disease.[1] A number of studies using raw forms (e.g., bulbs, cloves, fresh aqueous extracts), oils, and other preparations of both species have demonstrated antiplatelet activity (AA) *in vitro*[2−10] and *in vivo*.[1,11−17] In addition, several compounds, mostly organosulfur compounds, isolated from both species have demonstrated *in vitro* antiplatelet activity (IVAA).[18−21] The latter compounds are formed when fresh *Allium* tissues are crushed and cytoplasmic precursors, collectively called *S*-alk(en)yl-L-cysteine sulfoxides (ACSOs), are cleaved by the enzyme alliinase (EC 4.4.1.4), which is located in the vacuole.[22] Alliinase converts ACSOs to the respective sulfenic acids, pyruvate, and ammonia. Sulfenic acids are highly unstable and condense, in pairs, to form thiosulfinates (TSs) (for a comprehensive review on *Allium* biochemistry, see ref 23). Because pyruvate is also produced when TSs are formed, pyruvate content analysis is commonly used as an estimator of the total TS content.[24] The antiplatelet properties of *Allium* species are attributed to these sulfur compounds formed after alliinase-mediated cleavage of the ACSOs and subsequent reactions.[20]

AA in raw garlic and onion has been clearly demonstrated. However, worldwide, most of these bulbous vegetables are generally cooked before consumption. Recently, a study was conducted to evaluate the IVAA of whole and crushed garlic that were heated using different cooking methods and intensities.[25] Because *Allium* sulfur antiplatelet compounds are formed only after disruption of the bulb tissues, the heat treatments acted on different substrates involved in the antiplatelet response; in whole cloves of garlic where no TSs were yet formed, the heat could affect alliinase activity or the ACSOs, whereas in previously crushed garlic containing antiplatelet compounds (e.g., TSs), the heat acted on the latter compounds. The authors found that, although heating was, in general, detrimental for garlic IVAA, the extent of this effect varied among the different treatments used, ranging from unaffected AA (equal to raw garlic) to an absolute lack of AA, mainly depending upon how garlic was prepared (crushed or uncrushed) and the intensity of the heat treatment. Their results suggest that crushing garlic before moderate cooking can reduce the loss of activity (as compared to whole garlic) and that TSs are the main contributors to the antiplatelet response.

Whether these results are extrapolable to other *Allium* species has not been evaluated. *Allium* species vary in the type and content of their ACSOs and, consequently, in their TS profiles,[23] and differences in the physical and biochemical properties of alliinases from different *Allium* species have been found.[26−28] Moreover, onion but not garlic has lachrymatory factor synthase (LFS) enzymes, which convert the alliinase-produced sulfenic acids, namely, 1-propenyl sulfenic acid, into the *syn*-propanethial *S*-oxide, the lachrymatory factor (LF) responsible for inducing tearing when raw onions are chopped.[29] It is further thought that the reaction between the LF and sulfenic acids lead to the

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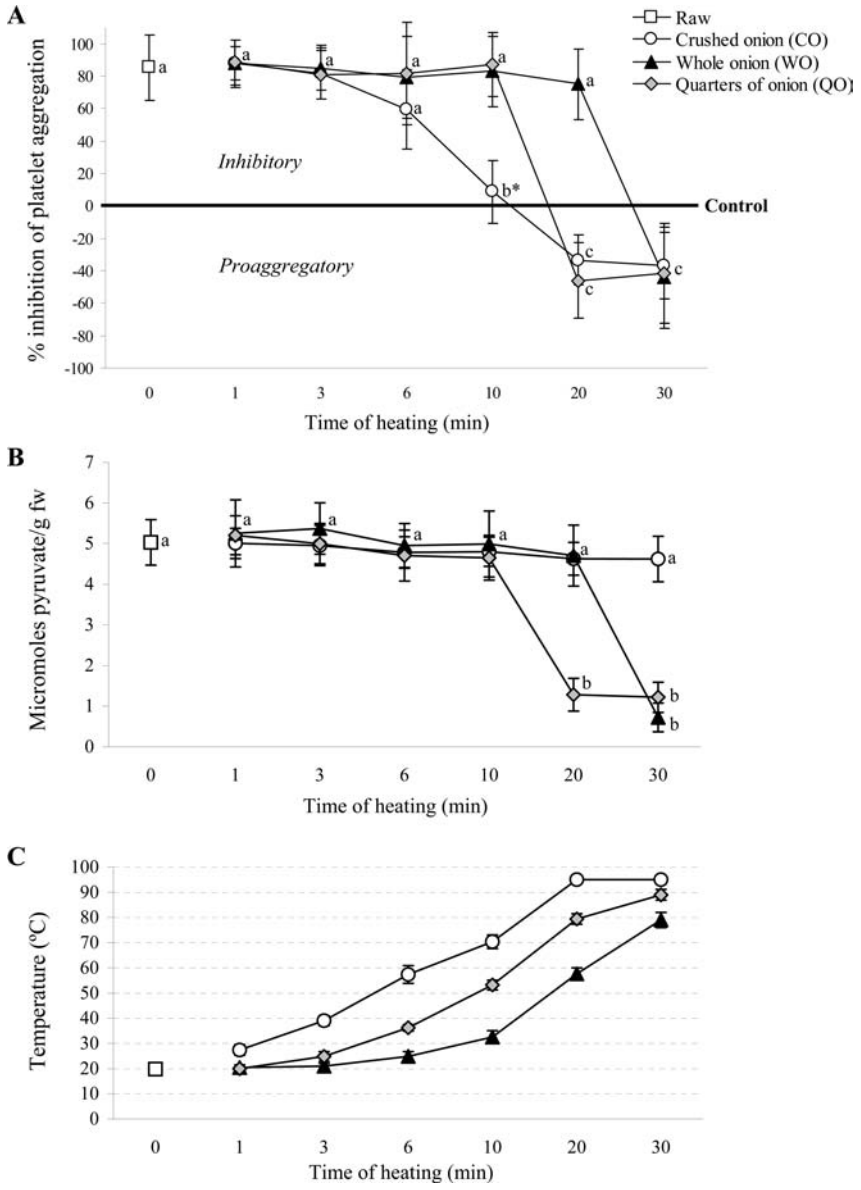

Figure 1. (A) IVAA, (B) pyruvate concentration, and (C) temperature reached in oven-heated onion samples of whole bulbs (▲), quarters of bulbs (gray ◆), and crushed bulbs (○). Values for IVAA are percent inhibition of platelet aggregation ± SD (n = 3−5). Asterisks indicate samples that are not significantly different from the control; $p < 0.05$. Values for pyruvate content (B) and temperature (C) are the mean ± SD (n = 3 and 5, respectively). Data points with no common letters differ; $p < 0.05$.

antiplatelet cepaenes and other sulfur compounds.[23] Therefore, it is possible that the results obtained in garlic are not extrapolable to other *Allium* vegetables.

The aim of this study was to evaluate the effect of domestic cooking on onion-induced antiaggregatory activity of human blood platelets and to compare the results to those reported previously in garlic.[25] For this, different cooking conditions and intensities were simulated, and the degradation of organosulfur antiplatelet compounds during the heat treatment was monitored by analysis of pyruvate levels. The efficacy of *Allium* species as antiplatelet agents, as affected by preparation and cooking conditions, is discussed.

MATERIALS AND METHODS

Plant Material. The onion cultivar "Cobriza INTA"[30] from the germplasm collection of INTA La Consulta, Mendoza, Argentina, was used in all of the experiments. Cobriza INTA is a pungent variety with a medium−high IVAA, as compared to other Argentine cultivars.[31] The onions were field-grown at the experimental station of INTA La Consulta, Mendoza, Argentina (33° 44′ S, 69° 07′ W), during 2005−2006 using standard agricultural practices. The bulbs were processed and analyzed between 30 and 60 days after harvest.

Processing of Samples. Two cooking methods, convection and microwave oven, were tested on onion preparations that varied in the extent to which bulb tissues were disrupted. Whole bulbs, quarters of bulbs, and completely crushed onions were used for the convection oven experiment, whereas whole and crushed bulbs were used for microwave heating.

In all cases, the starting material consisted of mature unsprouted bulbs of 110 ± 10 g, from which the outer dry leaves were removed. For the "bulb quarters" treatment, 3 kg of onions were cut in quarters longitudinally, the quarters were then mixed to avoid interbulb variation, and three samples of six quarters each were prepared. The samples were incubated at room temperature for 20 min to allow for enzymatic lyses of the ACSOs. For the crushed-tissue treatment, 3 kg of bulbs were crushed using a Philips HR 7633 food processor, homogenized, and incubated at

room temperature for 20 min. Samples of 150 g of the processed onions were prepared. After incubation, both types of samples, quarters and crushed onions, were heat-treated in a convection oven or by microwaves, as described in the following section. Whole bulb samples (consisting of 1 bulb/sample) were treated the same way, but without the incubation step. A total of 3 kg of fresh bulbs were crushed, mixed, juiced, and used as raw controls. For both cooking systems, different heating times were applied to the onion samples. In total, 27 different combinations (i.e., 27 treatments) of the cooking method, time, and tissue type were used. The entire experiment was repeated twice, resulting in 3 replicates per treatment.

Convection Oven. A convection gas oven (Longvie 2600) was heated to 200 °C. Whole onion (WO), quarters onion (QO), and crushed onion (CO) samples were placed in the oven in individual dishes and were heated for 1, 3, 6, 10, 20, and 30 min. At each time, one randomly selected sample of each tissue type (WO, QO, and CO) was removed from the oven and rapidly cooled by immersion of their containers in a water−ice mixture to stop the heat treatment. Individual samples were then juiced in 1 volume of distilled water (w/v) using a blender (Braun MR 555 CA Minipimer Control Plus Vario) and centrifuged twice. The supernatants were aliquoted and stored at −80 °C until IVAA and pyruvate analyses were performed.

Microwave. Samples of whole and crushed onions were microwaved individually in a Whirpool VIP 27 microwave oven at 500 W for 0.75, 1.5, and 3.0 s/g of fresh weight (fw). After microwaving, samples were cooled and juiced, as described.

Measurement of Platelet Aggregation. Determinations of IVAA were performed using electrical impedance aggregometry (Chrono-Log, Havertown, PA) of whole blood, as described before.[25] Briefly, onion juice samples were thawed at room temperature and homogenized by vortexing, and 220 μL was used in a standard aggregation reaction, which included 1 mL of blood and 2.5 μL of the agonist collagen (1 μg/μL). This volume of onion extract was chosen on the basis of near-zero aggregations (or ∼100% inhibition of aggregation) obtained for its raw form (control). For this, increasing doses of raw onion juice were assayed in the platelet aggregation reaction, resulting in 220 μL of onion juice being enough to produce complete inhibition of platelet aggregation *in vitro*. AA was expressed as percent inhibition of platelet aggregation, relative to control samples prepared in the same way but without the addition of onion juice. Proaggregatory effects were considered if values of electrical impedance in the "sample" cuvette containing onion extract were higher than in the "control" cuvette (without the addition of onion juice).

Blood was drawn from two healthy, non-smokers, human donors, one female and one male of ages 25 and 33, respectively, who had abstained from eating *Allium* species or other known platelet-inhibitory foods for at least 1 week. Also, the donors had not taken drugs known to affect platelet aggregation (e.g., aspirin) or other types of drugs for at least 1 week prior to venipuncture. The Institutional Board at the National University of Cuyo (Mendoza, Argentina) approved the protocol, and subjects signed informed consent prior to participation. In all cases, blood was drawn from fasting donors between 7 and 8 a.m. Venipuncture was performed by the hematology service at the Hospital Central, Mendoza, Argentina. Blood was drawn into citric acid anticoagulant and was later diluted 1:1 with Tris-buffered saline (pH 7.4). Day-to-day variations in the blood samples were checked and controlled as follows. For each blood sample, reference onion juice samples (of known AA) were tested for their IVAA, relative to a control sample without adding onion juice. If the IVAA of the reference samples was significantly different from the expected IVAA values, the blood sample was discarded.

Pyruvate Analysis. The pyruvate content was determined according to the method by Schwimmer and Weston.[32] Color development was measured at 420 nm on a spectrophotometer, and the pyruvate concentration in the juice was determined on the basis of calibration curves obtained by measuring pyruvate standards. Values were expressed as micromoles of pyruvate per gram of fw of onion. Because of its minimum contribution to the overall pyruvate content,[31] background pyruvate levels were not estimated.

Temperature Measurements. Multiple additional samples of WO, QO, and CO were prepared and heated using the same methodology and cooking systems as described above. The entire heating experiment was repeated 5 times (5 replicates/treatment). At each heating time interval, the temperature in the center of the bulbs (WO) and quarters (QO) and in the middle of the mass of crushed bulbs (CO) was measured using a YSI Tele-thermometer (Yellow Spring, Co., Yellow Springs, OH). Mean temperature values were calculated for each treatment of tissue time method.

Statistical Analysis. All data were expressed as the mean ± standard deviation (SD). The data were analyzed by the analysis of variation (ANOVA) procedure using the software Statgraphics Plus for Windows 4.0. Means of each treatment group were compared by the least significant difference (LSD) test. p values <0.05 were considered to be significant. Regression analysis between IVAA and pyruvate content was performed using the same software.

RESULTS

Convection Oven. Aqueous extracts of oven-heated onions varied significantly ($p < 0.001$) in their ability to inhibit platelet aggregation (Figure 1A). Heating of whole onions at 200 °C for 20 min or less had no significant effect on platelet aggregation, as compared to raw onion. However, when the heat treatment was applied for 30 min, WO samples had proaggregatory effects. Onion quarters heated for 10 min had IVAA comparable to that of the raw control but showed stimulatory effects on platelet aggregation after 20 min of heating. QO samples heat-treated for 20 and 30 min had the same proaggregatory response. Both types of samples with undisrupted bulb tissues (WO and QO) showed an abrupt decrease in IVAA during the heat treatment, varying from nearly complete inhibition of aggregation (comparable to raw onion) to having platelet proaggregatory effects. Completely crushed onions, on the other hand, showed a more gradual and sooner decrease in IVAA (Figure 1A). Although during the first 6 min of heating no significant lost in IVAA was observed (as compared to raw onion), crushed onions heated for 10 min had lost all AA (i.e., they were not statistically different from the negative control; $p < 0.05$), and after 20 min of heating, they were proaggregatory.

Pyruvate analysis reflects total TS content in *Allium* species, regardless of the type of TS. The pyruvate content varied significantly ($p < 0.001$) among oven-heated samples, with values ranging from 5.37 to 0.72 μmol of pyruvate/g of fw (Figure 1B). Heating for up to 30 min had no effect on the pyruvate levels of previously crushed onions, because they had the same content as the raw samples. Whole bulbs heated for 20 min or less were unaffected in their pyruvate levels, but their content decreased significantly (from 5.03 to 0.72 μmol/g of fw) when the samples were heated for 30 min. QO samples heated for ≤10 min had similar pyruvate content as raw onion, whereas QO samples heated for 20 and 30 min had significantly lower concentrations.

The rate at which temperatures increased inside the onion tissues during the heat treatment was associated with the degree of tissue disruption (Figure 1C). Crushed, quarters, and whole onion samples reached temperatures ≥50 °C after 6, 10, and 20 min of heating, respectively. The highest temperature measured in the onion samples during the experiment was 96 °C (which is also the temperature for boiling water in the location where the study was conducted), and only the CO samples reached this temperature.

Microwave. Microwave heating of whole and crushed onions significantly reduced IVAA in all of the samples (as compared to raw onion), regardless of the time used (Figure 2A). All microwaved whole onion (MWO) and microwaved crushed

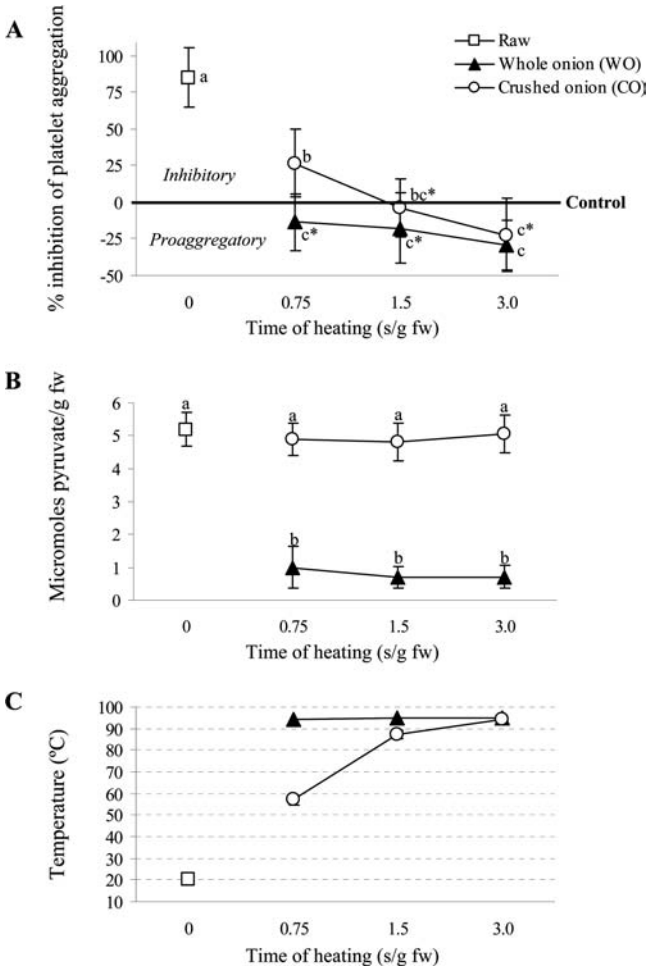

Figure 2. (A) IVAA, (B) pyruvate concentration, and (C) temperature in microwave-heated samples of whole (▲) and crushed (○) onions. Values for IVAA are percent inhibition of platelet aggregation ± SD (n = 3−5). Asterisks indicate samples that are not significantly different from the control; $p < 0.05$. Values for pyruvate content (B) and temperature (C) are the mean ± SD (n = 3 and 5, respectively). Data points with no common letters differ; $p < 0.05$.

onion (MCO) samples from the two most intensive heat treatments (1.5 and 3 s/g of fw) had no IVAA. Only crushed onions microwaved for 0.75 s/g of fw had some antiaggregatory activity (32% of the raw control).

Significant variation in the pyruvate content was found between whole and crushed microwaved onions but not among the different heat treatments within each tissue type (Figure 2B). MCO samples had pyruvate levels comparable to those of raw onions, with their content being approximately 6-fold higher than in the MWO samples.

The temperatures reached in the onion tissues after the microwave treatment were highest (∼95 °C) in whole onions, without noticeable differences among the microwaving times used (Figure 2C). On the other hand, the average temperature in crushed onion samples was 57 and 87 °C for the lowest (0.75 s/g of fw) and middle (1.5 s/g of fw) microwave intensities, respectively. MCO samples reached similar temperatures to MWO (95 °C) only at the highest microwave intensity (3 s/g of fw).

DISCUSSION

The results from this work indicate that heating may affect onion AA to different extents, depending upon the intensity of the heat treatment, the cooking method, and the way in which onion bulbs are prepared prior to heating (e.g., whole or crushed). The data from the convection oven experiment showed that crushed onions lost their IVAA before QO and QO lost their IVAA before WO (Figure 1A), suggesting a direct association between the degree of tissue disruption and the rate of degradation of antiplatelet compounds. Conversely, in a previous work using garlic cloves, we found that crushed garlic retained AA for a longer time than uncrushed cloves when the samples were heated in a convection oven at 200 °C or by immersion in boiling water.[25] These differences between both studies are mainly due to a much longer retainment of AA in whole onions (20 min, this work), as compared to whole garlic cloves (3 min[25]), because their respective crushed samples had a similar behavior with regard to IVAA as affected by heating (i.e., both crushed onions and crushed garlic lost their IVAA gradually between 6 and 10 min of heating in a convection oven or by immersion in boiling water). The analysis of temperatures in the onion tissues during the heat treatment and the pyruvate formed in their respective aqueous extracts provided an explanation for the observed differences.

In a convection oven (and also when heating by immersion in boiling water), the heat is transmitted from the outer tissues to the center of the bulb. Thus, at the beginning of the heat treatment, there is an important decreasing gradient of temperatures (Δt) between the outer layers and the center of the bulb. As heating time increases, the temperature inside the bulb rises and Δt becomes progressively smaller, tending toward 0. The rate at which this process takes place is bulb-size-dependent. In other words, larger bulbs require more heating time to reach a certain inner-bulb temperature than smaller bulbs. This is important because alliinase is heat-sensitive (according to Jansen et al.,[33] incubation of alliinase at 50 °C during 10 min inactivated 80% of the enzyme) and the production of antiplatelet compounds (e.g., TSs) in uncrushed heated *Allium* species depends upon the active alliinase that remains in the bulb tissues after the heating process.

Thus, when oven heating whole cloves of garlic, with an average size and weight of ∼20- and 70-fold smaller than the onion bulbs used in this study, respectively, the heat is rapidly transmitted to the entire clove tissues, inactivating the enzyme (between the third and sixth minute of heating) and, therefore, impeding the formation of TSs when the extracts are prepared. Conversely, in large onion bulbs, the most inner tissues reached alliinase-inactivating conditions (≥50 °C for 10 min) only after 30 min of heating (Figure 1C), and this was associated with a complete lost of AA and a sudden 7-fold decrease in pyruvate content (panels B and C of Figure 1). Accordingly, in the case of quarters of bulbs, alliinase-inactivating conditions were met after 20 min of heating (Figure 1C), and these conditions were also associated with a complete lost of AA and a marked decrease in pyruvate content (panels B and C of Figure 1). Presumably, cutting the bulbs in quarters increased the total specific surface area and reduced the volume of each unit, facilitating a more rapid heat transfer to the inner bulb tissues, leading to an earlier alliinase inactivation and, consequently, a complete lack of IVAA in the extracts. These results together with those reported previously for oven-heated and boiled uncrushed garlic (i.e., the lost of IVAA was markedly abrupt and complete and always

associated with a dramatic decrease in pyruvate and allicin contents[25]) strongly suggest that the observed loss of IVAA in intact *Allium* tissues is due to alliinase inactivation by heat, which impedes the formation of alliinase-derived sulfur antiplatelet compounds.

The time course variation of IVAA in intact onion tissues ("whole" and "quarters") during oven heating was markedly abrupt, varying from having the same inhibitory activity as the raw extracts ($\leq$20 and $\leq$10 min for WO and QO, respectively) to having no effect on aggregation at all ($\geq$30 and $\geq$20 min for WO and QO, respectively) (Figure 1A). The invariably high AA of the former samples, with AA similar to raw onion, can be explained by the fact the alliinase is present at a high concentration in the bulbs of both garlic and onion,[26,27] and therefore, only a small amount of active alliinase (e.g., from the inner bulb tissues, which have lower temperatures) is necessary for the full conversion of the ACSOs when the samples are juiced. In garlic, the addition of 10% of raw extract (with active alliinase) to an alliinase-free extract with uncleaved ACSOs resulted in the conversion of most if not all of the ACSOs, yielding the same TS content and IVAA as the raw extracts.[25]

The pyruvate content of oven-heated "whole" and "quarter" onions varied concomitantly with IVAA (panels A and B of Figure 1), and a strong positive correlation between both variables was found (r = 0.99; p < 0.001), suggesting that alliinase-derived antiplatelet compounds are responsible for the IVAA of onion extracts.

In crushed onions, the antiplatelet agents (e.g., TSs) were formed prior to the heat treatment. In agreement with previous results obtained with crushed garlic, these samples showed a gradual decay in IVAA. Presumably, the lost of AA in crushed onions during the heat treatment was due to the progressive destruction of antiaggregatory compounds, because it was strongly suggested in crushed garlic by the fact that allicin, the major garlic TS, showed a concomitant decrease with IVAA (r = 0.88; p < 0.001) during heating.[25]

The fact that crushed onions had lost all AA after 10 min of oven heating but conserved pyruvate levels comparable to that of raw onions, even for up to 30 min of heating, suggests differential heat sensitivities for these two compounds. Thus, once thiosulfinates and pyruvate are formed upon disruption of alliinase-containing tissues, degradation of TSs (i.e., the bioactive antiplatelet compounds) occurs more rapidly than degradation of pyruvate. Similar results were reported previously for garlic.[25]

Under our experimental conditions, microwave heating had a more severe effect on the IVAA of onion samples than heating in a convection oven. Whole and crushed onions lost their entire IVAA after 1.875 min (0.75 s/g of fw × 150 g of fw = 112.5 s = 1.875 min) and 3.75 min (1.5 s/g of fw × 150 g of fw = 225 s = 3.75 min) of microwave heating at 500 V, whereas in a convection oven, a comparable effect on IVAA was attained after 30 and 10 min of heating, respectively. This is likely due to the different conditions used in both cooking systems, which may represent different intensities of heating (i.e., it is possible that the combinations of voltage/time used in the microwave oven represent a more intense heat treatment than the conditions used in the convection oven experiment).

The longer retainment of antiaggregatory properties in whole versus crushed onions, as found in oven-heated samples, was not observed in the microwave assay. Furthermore, at the lowest microwave intensity (0.75 s/g of fw), whole onions had no IVAA, whereas crushed onions still retained some yet significant IVAA (Figure 2A). These results agree with those reported previously in garlic,[25] which suggest that sulfur antiplatelet compounds produced in crushed onion samples before the heat treatment (e.g., TSs) are more tolerant to heat than the enzyme alliinase (in uncrushed bulbs). The fact that microwaved whole onions rapidly reached alliinase inactivating temperatures (with the consequent lost of IVAA), as opposed to the results obtained in the convection oven, is probably due to the fact that microwaves act more uniformly[34] on the entire bulb tissues; therefore, $\Delta t \approx 0$, leaving no active alliinase for the conversion of ACSOs when the onions are juiced.

In the present study, collagen was used as a platelet agonist because it has been extensively used and validated by comparisons to other agonists for *Allium* AA determinations.[3−5,8,14,21,25,32,35] Several reports comparing garlic inhibition of platelet aggregation induced by different agonists, including adenosine 5′-diphosphate (ADP), collagen, arachidonic acid, epinephrine, and calcium ionophore A23187, have demonstrated comparable results among agonists; i.e., all agonists showed garlic inhibition of platelet aggregation in a dose-dependent manner for all of the agonists evaluated.[3,4,36,37] These results suggest that garlic antiplatelet compounds exert their effects at various stages involved in the process of platelet aggregation. Evidence from different biochemical and genetic analyses strongly suggest that thiosulfinates are the main contributors to garlic and onion AA.[8,20,21,38,39] Thus, assuming that thiosulfinates are the main antiplatelet agents in onion[8,21,38,39] and considering results from previous studies that compared different agonists for *Allium* IVAA, similar results can be expected in our study if other agonists were used.

We observed significant proaggregatory effects in 20 and 30 min oven-heated samples and in the most intense microwave treatment for crushed onions, suggesting that extensively cooked onions may stimulate rather than inhibit platelet aggregation. Normal hemostasis relies on the balance between procoagulant and anticoagulant activities. Onion bulbs contain compounds other than sulfur constituents with either AA (e.g., polyphenols, such as flavonoids) or procoagulant activity (e.g., prostaglandins) that may influence platelet function.[40] Presumably, if platelet-stimulant compounds present in onion were relatively more tolerant to heat than antiaggregatory compounds, prolonged exposure to high temperatures would result in a balance favorable for procoagulant activities.

An apparent platelet proaggregatory response as measured by *in vitro* light transmittance aggregometry has been reported for freshly juiced onion extracts (ca. 5 min post-juicing).[41] This effect was observed in agonist-free platelet aggregation reactions, and it was initially associated with the presence of ADP in the onion juice because ADP-free extracts, previously treated with the ADP scavenger enzyme apyrase, did not reveal the typical increase in light transmittance, indicative of platelet aggregation. However, the fact that platelet aggregates could not be observed under the microscope during the increase of light transmittance in the agonist-free reaction suggests that this response is associated with changes in light transmission through platelet-rich plasma rather than associated with platelet aggregation. Thus, according to the authors, the observed effect appears to be an artifact of the analysis when using freshly juiced extracts for light transmittance aggregometry. Such an effect is reduced to a minimum or disappears if the fresh extracts are allowed to stand at 25 °C for 30 min.[41] In the present study, the fact that onion extracts were "aged" at room temperature (~25 °C) for at least 20 min, together with the use of impedance aggregometry,

instead of light transmittance aggregometry, assured avoidance of this potential analytical artifact.

Stimulatory effects on platelet aggregation have been reported previously for boiled Welsh onions (*Allium fistulosum*),[42] onions heated by immersion in boiling water,[43] and raw extracts of different organs of the onion plant.[35] All of these studies used *in vitro* platelet aggregation assays. Whether these results are extrapolable to *in vivo* conditions needs to be investigated.

In summary, the present study provides insight into the mechanism by which heating may affect onion platelet antiaggregatory activity. In this regard, our results are in full agreement with those reported earlier in garlic. Together, both studies point to the importance of carefully examining the manner in which *Allium* vegetables are prepared and consumed, because these factors can significantly affect their efficacy as antiplatelet agents. We conclude that to obtain the maximum AA, onions and garlic should be crushed and eaten raw.

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Notes

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ABBREVIATIONS USED

IVAA, *in vitro* antiplatelet activity; AA, antiplatelet activity; TS, thiosulfinate; ACSO, *S*-alk(en)yl-L-cysteine sulfoxide; WO, whole onion; QO, quartered onion; CO, crushed onion

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