Low Allicin Release from Garlic Supplements: a Major Problem Due to the Sensitivities of Alliinase Activity

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Most garlic supplements are standardized on allicin potential and are enteric-coated to prevent gastric acid inactivation of the allicin-producing enzyme, alliinase. To determine whether these products release the claimed amount of allicin under simulated gastrointestinal conditions, USP dissolution method 724A for drug release was applied to all 24 known brands of enteric-coated tablets. It was found that nearly all brands employed effective coatings and that they met their claims for allicin potential when crushed and suspended in water. However, all brands except one gave low dissolution allicin release, with 83% of the brands releasing less than 15% of their potential. The low allicin release was found to be due to both impaired alliinase activity, mostly caused by tablet excipients, and to slow tablet disintegration, which also impairs alliinase activity. Only when tablets had high alliinase activity and disintegrated rapidly did they show high allicin release. The ability of USP 724A to estimate allicin release in vivo was validated by monitoring breath levels of the allicin metabolite, allyl methyl sulfide. In conclusion, garlic powder supplements should no longer be standardized on allicin potential, but rather on dissolution allicin release.

INTRODUCTION

Allicin (2-propenyl 2-propenethiosulfinate) has long been recognized as the main antimicrobial agent of crushed garlic (*Allium sativum* L.) cloves (*1*). More recent studies have provided strong evidence that it is also essential to most of the hypolipidemic effects of garlic, and much of the antithrombotic, antioxidant, and anticancer effects of garlic also appear to be due to allicin (*2*). In fact, no compound outside the thiosulfinates (of which allicin is about 75%) has yet been found that accounts for a significant portion of the pharmacological activities of crushed garlic, at levels representing normal human consumption (2−5 g/day). Consequently, the majority of the garlic supplements sold today are garlic powder tablets (some are capsules) that are standardized on allicin.

However, garlic supplements that intend to deliver allicin to the body must be prepared with considerable care because allicin is not actually present in garlic or in any garlic supplement. Allicin must be enzymatically formed by the action of the abundant garlic enzyme, alliinase (alliin alkyl-sulfenate-lyase EC 4.4.1.4), upon garlic's most abundant sulfur compound, alliin (L-(+)-*S*-allylcysteine sulfoxide) (Scheme 1). Allicin formation is easily accomplished when consuming raw garlic because its formation is complete within 6 s after cloves are crushed or chewed (*3*). However, allicin formation from supplements can be a significant challenge because alliinase activity is immediately and irreversibly destroyed below pH 3.5, the usual range for gastric juice (*3−5*). Even at neutral pH, its activity has been shown to decline rapidly at body temperature (*4*). Furthermore,

Scheme 1. Formation of Allicin from Alliin upon Crushing Garlic
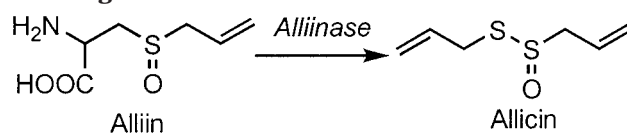
the processing of garlic into a powder, as well as the presence of tablet excipients, may decrease alliinase activity substantially, although the effects of these influences have not yet been reported.

Recognizing the importance of protecting alliinase from stomach acid, a large number of U.S. companies (64% of the tablet brands currently sold) market allicin-standardized enteric-coated garlic powder tablets. Enteric coatings are cellulose or poly(acrylic acid) esters that are acid-resistant but readily dissolve at neutral pH. While an enteric coating can effectively protect alliinase from stomach acid, the ability of such tablets to produce allicin under gastrointestinal conditions remains unknown, because of the possible influence of other factors on alliinase activity. Therefore, it is critical that allicin release (actually, formation and release) from garlic tablets be measured under simulated gastrointestinal conditions. Ideally these conditions should be validated in vivo with human subjects. The only officially recognized test for gastrointestinal release of compounds from enteric-coated tablets are those defined by the U.S. Pharmacopeia (USP) for dissolution of delayed-release tablets (Method 724A) (*6, 7*). Method 724A is a general method that requires protection of tablet constituents from dilute acid (0.1 N HCl) for 2 h and subsequent release of 75−80% of the claimed drug content in 45 min in a pH 6.8 buffer, although extension to 60 min in buffer for allicin release is currently under review (*7*). Any garlic supplement that claims to release allicin, whether enteric-coated or not, needs to be

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considered as a delayed-release product, because alliinase must be protected from gastric acid. This method has already been applied to two brands of nonenteric-coated tablets that have been used in failed clinical trials on serum cholesterol reduction, with the finding that the dissolution allicin release was very low: 0–15% of expected values (*8*).

The objective of this study was to employ USP Method 724A to evaluate the allicin-releasing ability of all known brands of enteric-coated garlic tablets sold in the U.S. and to determine if any deficiencies could be due to impaired alliinase activity. Furthermore, the in vivo validity of USP 724A was examined by monitoring breath levels of allyl methyl sulfide, the main metabolite of allicin (*2, 9*).

MATERIALS AND METHODS

Allicin Release and Tablet Dissolution/Disintegration. Almost all products were purchased in late 1998 or 1999 from local vendors, and all were tested well within their expiration dates. The release of allicin from the tablets was determined according to the general drug release standard for delayed-release articles (USP Method 724A) (*6*). Using a model VK6010 dissolution apparatus (VanKel Technology Group, Cary, NC) equilibrated at 37 °C, one tablet was placed into a covered 1-L round-bottom glass vessel containing 750 mL of 0.1 N HCl and paddle-stirred at 100 rpm for 2 h, after which 250 mL of 0.2 M Na_3PO_4 was added and the pH was slightly adjusted if necessary, giving 1000 mL at pH 6.80 ± 0.05. After the solution was stirred for 45 min and other indicated times in the intestinal buffer, 1 mL of medium was added to 0.05 mL of 210 mM carboxymethoxylamine (final 10 mM) (Sigma, St. Louis MO) for allicin determination. Carboxymethoxylamine is a highly efficient alliinase inhibitor (*10, 11, 13*). Usually six or more tablets were tested for each brand. The general method does not specify a stirring speed, but defers to individual drug monographs for delayed-release tablets, all of which specify 100 rpm, including the currently pending monograph on garlic tablets (*7*). The pending monograph is nearly identical to the general method except that the buffer time is extended to 60 min and the stirring speed is given. The time to achieve complete disintegration was determined by observation during the dissolution test.

Allicin Synthesis. Allicin was prepared in high purity (98%) by oxidation of diallyl disulfide by modification of previous methods (*11–13*). One gram of commercial diallyl disulfide (about 80% pure, Aldrich, Milwaukee, WI) was fractionally distilled to 98% purity and dissolved in five mL of cold (4 °C) glacial acetic acid, to which 1.5 mL of cold 30% hydrogen peroxide was slowly added. After 30 min, the temperature was allowed to increase to 12–15 °C and stirring continued for 4–6 h until the diallyl disulfide content decreased by only 75–80% (prevents over-oxidation to diallyl thiosulfonate). The reaction was stopped with addition of 15 mL of water, and was extracted with 30 mL of dichloromethane. Acetic acid was removed by washing the extract several times with 5% $NaHCO_3$ and then washing with water to pH 6–7. After evaporation of the solvent, allicin was redissolved in 500 mL of water, and unreacted diallyl disulfide and other low-polar impurities were removed by double extraction with 0.1 vol of hexane. The final solution (1–2 mg/mL water) is stable for about 24 h at room temperature, 2 months at 4 °C, and for at least 2 years at −70 °C (*11, 14*). The yield was quantitated gravimetrically by extraction of a portion of the final solution with 2 volumes of dichloromethane and rotary evaporation to a constant weight, as well as by the extinction coefficient in water at 240 nm, 2380 M^{-1} (14.64 mL/mg) (*14*). The yield was almost quantitative based on the amount of diallyl disulfide depleted. The purity (weight %) was determined by reversed-phase HPLC using 75% methanol at 1 mL/min at 240 nm, using the extinction coefficient for ajoene, 8730 M^{-1} (37.3 mL/mg), the main impurity (elutes about 0.5 min after allicin) (*15*).

Allicin Analysis. Dissolution samples were assayed directly for allicin content. HPLC analysis was conducted with a 250 × 4.6 mm, 5 μm, LC-18 column (Supelco, Bellefonte, PA) eluted with methanol/water (60:40) at 1 mL/min and detected at 240 nm. An injection volume of 80 μL was used for all samples and for a diluted allicin standard. For alliinase activity, an injection volume of 30 μL was used.

Laboratory-Made Garlic Powders. Garlic cloves from the same bulb have been shown to vary only about 5% in allicin yield (*14*). Hence, to be able to make good comparisons, freeze-dried and oven-dried garlic powders were made from cloves taken from the same bulbs. Garlic bulbs were purchased at five different grocery stores on the same day. Average clove weights varied from 3.0 to 4.8 g, with many individual cloves reaching 5.5–7.0 g. From a single bulb, 3–4 cloves (about 20 g) were peeled, their tips were removed, and the remainder was cut into 3–4-mm-thick slices. The slices were mixed and weighed, and about 10 g each was placed in a freeze-dryer for 91 h or in a static oven at 55 °C for 50 h to constant weight. The dried slices were then pulverized for 1 min in a Braun coffee grinder and passed through a 355-μm (42 mesh) screen, 80% of which could pass through a 125 μm (120 mesh) screen. The remaining cloves from each bulb were set aside to be assayed as fresh garlic.

Allicin Potential. Allicin potential is the maximum achievable allicin yield when garlic powder or crushed (mortar-ground) tablets are added to water. Maximum values were found when crushed tablets were incubated in ambient water at 50 mL/g for 30 min after 15 s of shaking. This represents a large excess of incubation time because cloves and powders normally only need 6–30 s to reach maximum allicin at this concentration (*3, 11*). For crushed tablets this dilution (50 mL/g) gave slightly higher values in a few cases than at 25 mL/g and higher values in several cases than at 200 mL/g. For garlic powders, dilutions at 25–200 mL/g gave essentially identical results. The allicin potential cannot be defined on the basis of the alliin content of the tablets because the alliinase activity of some brands is so low that much of the alliin is never converted to allicin and because about 15% of the alliin is converted to other allyl thiosulfinates besides allicin (*14*).

Alliinase Activity. The alliinase activity of fresh garlic, garlic powders, and crushed tablets varies greatly with dilution. To be able to accurately compare the alliinase activity between tablet brands and between fresh garlic and garlic powders, the same dilution must be used. This dilution must also be based on the same amount of dry plant material, meaning (1) the actual weight of garlic powders, (2) the dry weight of fresh garlic, and (3) the weight of garlic powder inside the garlic tablets. The dilution that was chosen to determine the activity of garlic and garlic products was 800 mL of water per gram of dry weight or, for tablets, per gram of garlic powder content claimed. At this concentration, linear initial allicin formation rates, the basis of enzyme activity, could be determined for fresh garlic and all garlic products. At higher concentrations (such as 400 mL/g dry garlic), allicin formation was too rapid for some tablet brands and for all fresh garlic products and hence was already less than linear by 15 s, the lower time limit to be able to homogenize cloves or mix powders with water and remove aliquots to the alliinase inhibitor. At lower concentrations (such as 1600 mL/g), the allicin concentration became too difficult to detect for several tablet brands and spice powders.

Garlic tablets, 10 or more per brand, were ground with a mortar and pestle to a fine powder and passed through a 125-μm (120 mesh) sieve. Most garlic powders also required grinding to achieve the same fineness. The powders were added to ambient water at 800 mL/g garlic powder (not per gram tablet) and shaken vigorously for 15 s, then allowed to sit. At three time points, 1 mL of solution was added to 0.05 mL of 210 mM carboxymethoxylamine to stop the reaction. After microfuge centrifugation, the samples were assayed directly for allicin content. Powders with high activity were typically assayed at 20, 30, and 45 s; those with moderate activity were assayed at 30, 45, and 60 s; whereas those with lower activity were assayed at 60, 120, and 180 s or longer, if

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needed, to produce a detectable amount of allicin. Powders with very high activity (>15000 μg allicin/min/g) were shaken for 10 s and assayed at 15, 20, and 30 s.

Fresh garlic was peeled and a single slice of clove (0.7–1.1 g) was added to the amount of water needed to provide 800 mL/g dry weight (the % dry weight was determined on the basis of the weight loss for the dried preparations previously mentioned) and homogenized at the highest speed in a 500-mL Waring blender for 10 s. This was sufficient time to give complete homogenization, but not if a larger blender was used. Aliquots were added to inhibitor at 15, 20, and 30 s.

The linearity of the rate of allicin formation was evaluated on the basis of expected allicin ratios. For example, if the amount of allicin found at 30 s was about 50% higher than the amount found at 20 s, then the rate of allicin formation was considered linear through 30 s, and the allicin value that gave the highest rate of allicin formation, usually the shorter time period, was used as the alliinase activity value. If the ratio was significantly less than expected, then the sample was assayed at a shorter time period. For most samples, the first time period gave the highest activity value, but for some tablets, perhaps due to the influence of excipients, the highest activity values were found in the second or third assay aliquot. The aliquot that gave the highest activity was used to represent the alliinase activity of the sample. The alliinase activity for each assay time point was calculated as the allicin concentration (μg/mL) of the 1.05-mL assay aliquot times 800 times 1.05 divided by the assay reaction time (min).

Independent Dissolution Testing. Because this study was conducted with brands commercially competing with those produced by the company conducting the study, six of the 24 brands were sent to an independent laboratory (Sage Pharmaceuticals, Shreveport, LA) for verification of the reported allicin release, according to USP 724A at 100 rpm. The results were simultaneously sent to our laboratory and two other laboratories (Eric Block, Chemistry Department, SUNY, Albany, NY 12222 and Gary Abrams, Department of Medicine, University of Alabama, Birmingham, AL 32594).

Allicin Bioavailability. Allicin bioavailability was determined after consuming single doses of garlic powder tablets and pure allicin with a standard meal (280 g of sweetened yogurt), measuring the area under the 48 h curve for the plot of exhaled allyl methyl sulfide (μg/L) versus time. Allyl methyl sulfide is the main metabolite of allicin (*2, 9*). It is maximally produced 3–4 h after allicin consumption and is no longer detectable after 36–48 h. Pure neat allicin was freshly extracted from aqueous solution with dichloromethane, as described under Allicin Synthesis, and transferred to gelatin capsules. In neat form it was stable for at least 1 h at 23 °C and 5 h at 2 °C.

Whole breath samples were collected in 1.2-L Teflon bags (Alltech, Deerfield, IL) containing a septum port and a stainless steel valve, to which a 4-cm piece of Tygon tubing was added. Samples were taken every 1–2 h, except during sleep, and analyzed within 1–16 h. Duplicate 5-mL breath samples were injected directly into the gas chromatograph fitted with a 30 m × 0.32 mm × 0.25 μm Supelcowax 10 capillary column (Supelco, Bellefonte, PA), with a helium carrier gas flow rate of 3.2 mL/min and a split ratio of 20:1. The column was operated isothermally at 50 °C. The injection port and FID detector were operated at 225 °C and 250 °C, respectively. The allyl methyl sulfide (98%, Aldrich, Milwaukee, WI) vapor standard was prepared by transferring 4.00 μL to a 4.3 -L nitrogen-filled glass bottle. After waiting at least 20 min, 43 mL of vapor was transferred via a gastight syringe to a second 4.3-L bottle, giving a final concentration of 7.44 μg/L. After conducting the experiments it was found that alveolar breath, determined using an alveolar bag (Quintron, Milwaukee, WI) attached to the Teflon bag for exiting mouth and throat air, gave an 18% higher concentration of allyl methyl sulfide than whole breath. However, as only relative amounts were critical, this was not an important deficiency.

Statistical Analysis. Where indicated, values are expressed as means ± SD. Differences between groups were assessed using the one-tailed Student's *t*-test (Microsoft Excel).

Table 1. Tablet Descriptions and Allicin Potential of Enteric-Coated Garlic Powder Tablets Sold in the U.S.

brand	tablet wt (g)	garlic powder claimed (g/tablet)	allicin potential claimed (μg/tablet)	allicin potential found[a]		
				μg/tablet[b]	% of claim	mg/g powder[c]
A	0.68	0.35	3200	3930	123	11.2
B	0.97	0.50	5000	5410	108	10.8
C	1.01	0.65	6000	5380	90	8.3
D	0.83	0.40	- - -[d]	4700	- - -	11.8
E	0.49	0.32	5560	5450	98	17.0
F	0.56	0.50	750	1570	209	3.1
G	0.83	0.40	5000	4990	100	12.5
H	0.61	0.30	585	880	151	2.9
I	1.04	0.60	6000	7030	117	11.7
J	0.84	0.65	3900	5300	136	8.2
K	0.61	0.40	4000	4050	101	10.2
L	0.25	0.10	- - -[d]	380	- - -	3.8
M	0.78	0.40	3000	4030	134	10.1
N	1.21	0.45	5400	2100	39	4.7
O	0.80	- - -[d]	4450	5310	119	- - -
P	0.88	0.40	4000	3280	82	8.2
Q	0.99	0.60	5000	4770	95	8.0
R	0.82	0.60	3600	4640	129	7.7
S	0.98	0.40	2400	2990	125	7.5
T	0.74	0.40	- - -[d]	1740	- - -	4.4
U	0.59	0.18	5900[d]	1790	30	9.9
V	1.43	0.30	2400	2870	120	9.6
W	0.78	0.40	- - -[d]	4660	- - -	11.7
X	0.90	0.65	6500	6600	102	10.2

[a] The maximum amount of allicin that can be formed in water, determined when crushed tablets are incubated in water at 50 mL/gram tablet for 30 min at ambient temperature. [b] Values for μg/tablet are means of 2–4 analyses of single lots. Brands A, E, G, I, and K were tested for variation among 3–4 lots and were found to have the following coefficients of variation: 15%, 15%, 9%, 6%, and 10%, respectively. [c] Calculated as μg/tablet found, times 0.001, divided by the amount of garlic powder claimed. [d] No value claimed on label. For brand U, allicin claimed was calculated from the alliin claim.

RESULTS

Tablet Descriptions and Allicin Potential. Both the tablet weights (0.25 to 1.43 g) and the claimed amounts of garlic powder per tablet (0.10 to 0.65 g) were found to vary about 6-fold (Table 1). The mean content of garlic powder claimed in the products was 54 wt %. All products were tablets except brand H, which consisted of enteric-coated garlic granules in a gelatin capsule, and brands N and U, which were enteric-coated softgel capsules containing powder suspended in a vegetable oil. Brands E and U contained both garlic powder and dried extracts of garlic. All brands claimed to be enteric-coated, except for brands F, R, S, and V. Brand S listed an enteric-coating agent on the label, whereas brands F, R, and V performed in acid as if they were enteric-coated. All brands, except D, T, and U, made claims for allicin potential (or yield), two of which (L and W) made nonnumerical claims ("rich in allicin" and "far greater than other types"). Ten of the brands also claimed alliin content. Brand U claimed only alliin content.

The allicin potential claims varied from 585 to 6500 μg/tablet, but most brands varied from 2400 to 6500 μg, with a mean claim of 4520 μg. The actual allicin potential found (Table 1) agreed fairly well with the claims for most brands, with only two brands (N and U) being substantially below claim. However, both of these brands contained garlic powder suspended in vegetable oil, a condition that is known to result in a 50% loss per year of allicin potential, due to loss of alliin (*16*). Many brands had more allicin potential than

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Table 2. Dissolution Allicin Release (USP 724A) for Enteric-Coated Garlic Powder Tablets

			allicin release in intestinal buffer[b]			
			USP time limit		extended times[e]	
	tablet appearance after 2 h in	tablet disintegration time in buffer, after	0.75 h		2 h	≥3 h
brand	acid (% intact)	2 h in acid[a] (h)	μg/tablet[c]	%AP[d]	%AP	%AP
A	100	0.88	3690 ± 110 (12)	94	>98	>98 (3 h)
B	100	0.92	1200 ± 70 (5)	22	40	38 (3 h)
C	100	1.2	440 ± 270 (5)	8	29	29 (4 h)
D	100	3.0	510 ± 80 (6)	11	31	52 (4 h)
E	100	1.5	140 ± 15 (6)	3	7	7 (4 h)
F	blisters	3.0	<120 (5)	<8	<8	<8 (4 h)
G	100	4.0	350 ± 70 (6)	7	19	31 (4 h)
H	- - -[f]	- - -[f]	<120 (8)	<14	<14	<14 (10 h)
I	blisters	1.0	300 ± 50 (6)	4	11	11 (4 h)
J	100	1.2	310 ± 90 (6)	6	7	7 (10 h)
K	100	0.75	290 ± 60 (5)	8	12	12 (4 h)
L	100	7.0	<20 (10)	<6	<6	43 (7 h)
M	100	2.75	290 ± 40 (6)	7	28	65 (4 h)
N	100	0.75	260 ± 110 (5)	12	18	18 (3 h)
O	100	3.8	<120 (5)	<3	<3	4 (5 h)
P	100	7.0	150 ± 30 (6)	5	13	30 (9 h)
Q	blisters	3.0	200 ± 20 (8)	4	23	32 (4 h)
R	100	3.0	<120 (7)	<3	<3	<3 (6 h)
S	swollen	0.92	1020 ± 240 (12)	34	58	56 (4 h)
T	100	1.5	240 ± 110 (9)	14	49	52 (6 h)
U	100	9	<120 (7)	<7	<7	<7 (10 h)
V	100	>66[g]	<120 (5)	<5	<5	<5 (66 h)
W	100	6.0	380 ± 60 (6)	8	18	39 (20 h)
X	100	1.2	1150 ± 240 (6)	17	55	58 (3 h)

[a] The time required for complete disintegration of the tablets while using USP Method 724A. [b] The amount of allicin released from one tablet after 2 h in 0.1 N HCl and various times in phosphate buffer (pH 6.8) at 100 rpm according to USP Method 724A. Some products gave values (<) below the limit of detection of 120 μg/L or 120 μg/tablet. Brand L had a lower detection limit because six tablets were used in a single vessel because of their small size and small allicin potential. [c] Values for μg/tablet are for single lots expressed as mean ± standard deviation for (*n*) tablets, tested individually. Brand A was significantly greater (*P* < 0.0005) than all other brands, whereas brands B, S, and X were significantly greater (*P* < 0.001) than all brands with smaller values. Brands A, E, G, I, and K were examined for variability among 3−4 lots per brand and were found to have the following coefficients of variation: 4%, 14%, 14%, 61%, and 60%, respectively. [d] Percent of the allicin potential (AP) found, as reported in Table 1. [e] Allicin release found upon continuing the Method 724A incubation period at pH 6.8. [f] Product H consists of enteric-coated granules in a gelatin capsule; hence, the disintegration time could not be observed. [g] Observation of brand V was discontinued after 66 h because about 90% of the tablet was still intact.

claimed, but it is common to intentionally include excesses of 10−25% to allow for natural variation and gradual instability losses over time.

Dissolution Allicin Release. Tablet disintegration times and allicin release under the USP-defined simulated gastrointestinal dissolution conditions are detailed in Table 2. It was found that most brands had effective enteric-coatings, as there was no change in tablet appearance after 2 h in acid other than small blisters in a few cases. However, after the acid stage, most brands (83%) did not release even 15% of their allicin potential into the intestinal buffer. This is partly due to the slow disintegration of the tablets in buffer, as only six brands completely disintegrated by the USP-724A-specified dissolution time of 0.75 h or the pending specification for garlic-tablet dissolution time of 1.0 h. Half of the brands required 3 h or more to disintegrate, and several required 6 h or more. Extending the dissolution time in buffer to 2 h resulted in improved allicin release for some brands, but little improvement for many. Even after extending the dissolution time until complete tablet disintegration or longer (Table 2, last column), 79% of the brands still released less than 35% of their allicin potential. Only brand A (Garlicin, Nature's Way Products, Springville, Utah) met the USP requirement of releasing at least 80% of its label claim in 0.75 h (released 115% of claim). The next best brands released 43% (S), 24% (B), and 18% (X) of their label claims (cf. Table 1). Allicin itself was sufficiently stable for the duration of the dissolution testing, with no

detectable loss in 6 h during the buffer stage and a half-life of 90 h at 37 °C in water (not shown).

The results in Table 2 for allicin release at 0.75 h according to USP 724A were confirmed for samples (same lot numbers) of six brands by an independent laboratory (Sage Pharmaceuticals, Shreveport, LA). The results agreed within 7.3% for brand A (3420 μg/tablet). For brands E (66 μg/tablet), G (215 μg/tablet), I (210 μg/tablet), K (215 μg/tablet), and Q (180 μg/tablet) the results were less consistent but in reasonable agreement because measuring small values is less accurate.

As a partial explanation for the low allicin release values that were generally found, even after complete disintegration, it appears that alliinase activity is fully inactivated within a few minutes after tablet disintegration. This is shown by the brands that disintegrated in under 2 h (B, C, E, I, J, K, N, S, T, and X), which showed no improvement in allicin release between 2 h and ≥ 3 h. Furthermore, addition of crude alliinase (*8*) to the medium, after complete tablet disintegration (not shown), did produce the expected amount of allicin (>98% of AP), demonstrating that alliin was present. Hence, alliinase appears to be active only at the surface of the disintegrating tablet and not in the dissolution medium.

Tablet Alliinase Activity. To further examine the cause for the low allicin release from the tablets, even from those that disintegrated near the USP limit of 0.75 h (brands B, C, I, J, K, N, X), the alliinase activity of the pulverized tablets was measured. As shown in Table

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Table 3. Alliinase Activity of Enteric-Coated Garlic Powder Tablets

brand	tablet alliinase activity[a]	brand	tablet alliinase activity[a]
A	14400	M	3030
B	1500	N	110
C	680	O	2600[b]
D	5600	P	4800
E	1030	Q	1850
F	<100	R	720
G	3700	S	860
H	570	T	120
I	290	U	<100
J	320	V	860
K	1570	W	6900
L	470	X	320

[a] Values are μg of allicin formed min^{-1} g^{-1} garlic powder claimed, determined as the initial linear rate of allicin formation for finely crushed tablets. Values are means for 2–5 determinations of single lots of each brand. The coefficients of variation for 5 determinations of 6 brands, covering nearly the entire range of activity, were found to be A (6%), D (2%), G (18%), Q (3%), E (15%), and X (14%). All differences between these brands were highly significant ($P < 0.0005$). [b] The amount of garlic powder per tablet was not claimed for this brand, so 50% of tablet weight was assumed.

3, a large activity range of at least 140-fold was found, with 13 of the brands having low activity (<1000 μg allicin/min/g garlic powder), seven having very low activity (<400), and only three (A, G, W) having activity similar to fresh garlic (>5000) (Table 4). Of the brands that disintegrated by about 0.75 h in intestinal buffer and yet still gave low allicin release (B, C, I, J, K, N, X) (Table 2), all had alliinase activity values of <1600. Therefore, low tablet alliinase activity is also a major reason for the failure of many brands to form and release allicin.

Effects of Processing on Alliinase Activity and Allicin Potential. Because of the low alliinase activity found for most tablet brands, the effects of drying and commercial processing of fresh garlic were investigated. The powders used in most garlic supplements are typically prepared by drying garlic clove slices in low-temperature ovens (50–60 °C) or by freeze-drying. To examine the effects of these drying methods on the alliinase activity and allicin potential of fresh garlic, freeze-dried and oven-dried powders were prepared from the same individual bulbs as the fresh garlic. As shown in Table 4, freeze-drying did result in a 22% mean loss of alliinase activity, whereas drying at 55 °C resulted in a 48% loss. Freeze-drying did not cause a loss in allicin potential, but drying at 55 °C resulted in a 16% loss. The alliinase activity and allicin potential of four brands of commercial powders that are specifically made for garlic supplements and are standardized to a minimum allicin potential of 10–10.5 mg/g (Table 5) were similar to those of the powders prepared for Table 4. However, commercial spice garlic powders (Table 5) appear to be made by much harsher drying methods because they had a mean alliinase activity equivalent to only 3% that of fresh garlic or 4–6% that of the other garlic powders and an allicin potential of only about 30% that of fresh garlic or the other powders.

Five (A, D, G, P, W) of the 24 brands of tablets in Table 3 had alliinase activity levels that approached those of the lowest values for the freeze-dried or oven-dried powders prepared for Table 4. The activities of the other 19 brands were similar to those of the spice powders (Table 5), but only brands F, H, L, and T had

both alliinase activity and allicin potential that were similar to the spice powders, indicating that these four brands were made with spice quality powders. The allicin potential of all other brands of tablets (mean 10.0 ± 2.6 mg/g garlic powder, excluding F, H, L, T; Table 1) compared favorably to those of fresh garlic and the other powders (Table 4), indicating that most brands were made with high-quality garlic powders. Therefore, the large difference in alliinase activity between most tablet brands and the supplement-quality garlic powders must be due to factors involved in tablet making, particularly the added excipients, although temperature and pressure may also be involved.

In Vivo Validation of Dissolution Allicin Release Estimated by USP 724A. Four sets of enteric-coated tablets with different levels of dissolution allicin release were each consumed at the same allicin potential of 48 mg. All four sets of tablets were prepared in our facility and were very similar in weight, shape, garlic powder content, excipients content, and USP 724A disintegration time (45–53 min), but each set was made with a different batch of garlic powder. The powders varied about 2-fold in allicin potential, although this difference was compensated for by consuming different numbers of tablets (12-21 tablets). The main distinction among the powders and tablets was the level of alliinase activity. The tablet alliinase activity varied by 47-fold (200–9400 μg allicin/min/g garlic powder), resulting in an 18-fold variation in the tablet allicin release (215–3900 μg/tablet) (Figure 1). The set of tablets with the highest allicin release is brand A.

Comparison of the dissolution allicin release from the tablets by evaluating the allyl methyl sulfide exhaled by three persons (Figure 1) revealed a strong correlation between the two ($R = 0.966$ for person 1, 0.998 for person 2, and 0.991 for person 3). Importantly, the projected Y-intercept values are near zero in all cases. Low Y-intercept values indicate that dissolution allicin release gives a reasonably accurate estimate of in vivo allicin formation. Had the USP 724A standards for the dissolution conditions been too strict, that is, if more allicin had actually been produced in the body than predicted by USP 724A, then the Y-intercept would have been substantially higher. In fact, if all the sets of tablets had produced in the body all the allicin they are capable of, then the plots would have been parallel to the X-axis. The amount of allicin released by the highest set of tablets does represent complete allicin formation and release because consumption of an equivalent amount of pure allicin by persons 1 and 2 resulted in an amount of total exhaled allyl methyl sulfide (as AUC) of 8.5 ± 2.4 μg-h/L (not shown), which was not significantly different from consuming the tablets (9.1 ± 2.5 μg-h/L).

DISCUSSION

The results of this study clearly demonstrate that there are factors other than enteric coating and allicin potential that are critical in the design of garlic tablets that intend to deliver allicin to the body. Although the enteric coatings were found to effectively protect alliinase from stomach acid for at least 2 h, nearly all brands produced very little allicin under the simulated gastrointestinal conditions defined by USP method 724A. The main reasons for the low allicin release were low tablet alliinase activity and slow disintegration times. For a few brands the low tablet alliinase activity was

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Table 4. Alliinase Activity and Allicin Potential of Fresh Garlic and Powders Prepared from the Same Bulbs

		alliinase activity (μg allicin min^{-1} g^{-1} dry wt.)			allicin potential (mg/g dry wt.)		
store no.	bulb no.	fresh	freeze-dried[a]	dried at 55 °C[b]	fresh	freeze-dried[a]	dried at 55 °C[b]
1	1	43000	35000	25900	14.5	14.2	12.6
	2	34000	24800	17700	12.0	10.9	8.1
2	1	7800	7600	5500	11.0	11.0	9.2
	2	14000	11000	4200	10.4	9.4	9.1
3	1	8300	7900	4400	7.8	7.9	6.9
	2	7500	8400	4200	8.0	7.4	6.5
4	1	7500	7800	3600	10.5	12.4	6.8
	2	11300	7100	3200	10.5	9.9	9.1
5	1	30100	18300	14400	12.6	14.5	12.1
	2	27700	20400	16400	10.2	10.8	10.0
mean ± S. D.		19100 ± 12600	14900 ± 9600[c]*	9900 ± 7900*, **	10.8 ± 2.0	10.7 ± 2.4	9.0 ± 2.1*,**

[a] Powder prepared by drying sliced cloves from the same bulb in a freeze-dryer for 91 h. [b] Powder prepared by drying sliced cloves from the same bulb at 55 °C for 50 h. [c] *Significantly different from fresh garlic ($P < 0.005$), paired t-test. **Significantly different from freeze-dried garlic ($P < 0.005$), paired t-test.

Table 5. Alliinase Activity and Allicin Potential of Commercial Spice Garlic Powders and Powders Sold for Garlic Supplements

brand[a]	alliinase activity (μg allicin min^{-1} g^{-1})	allicin potential (mg/g)
	spice powders	
A	280	2.5
B	260	2.3
C	1800	5.3
D	180	2.7
E	470	3.4
mean ± S. D.	600 ± 680	3.2 ± 1.2
	supplement powders	
A	12300	10.4
B	9200	11.3
C	15200	12.9
D	9900	9.7
mean ± S. D.	11700 ± 2700	11.1 ± 1.4

[a] Spice powders were purchased at grocery stores. Supplement powders were obtained from commercial bulk vendors and are sold as standardized to a minimum allicin potential of 10–10.5 mg/g.
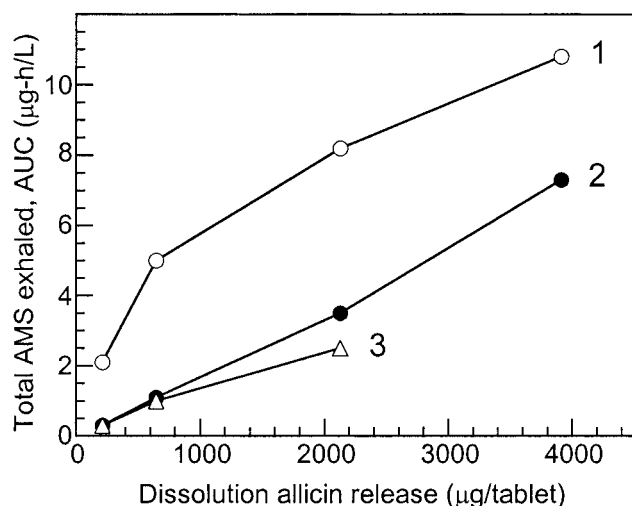

Figure 1. Correlation between the in vitro allicin release (USP 724A, 60 min intestinal buffer) of enteric-coated garlic powder tablets of increasing alliinase activity, but the same allicin potential, and the amount of allyl methyl sulfide (AMS) exhaled from three persons in 48 h after tablet consumption. Person 3 moved before completing the study. All tablets disintegrated by 55 min in the buffer.

due to using low-quality garlic powders with little alliinase activity, but most brands appear to have employed powders with high alliinase activity. However, the alliinase activity of the powders used in these brands was greatly impaired by factors associated with tablet making, presumably mostly by tablet excipients, although compression and heat may also be important.

There are two reasons why slow tablet disintegration times result in low dissolution allicin release from tablets. The first is simply due to the time limit of 0.75–1.0 h established in the USP method, because half of the brands required 3 h or more to disintegrate. The second reason is less obvious and is due to the natural rapid deactivation of activated alliinase. Since the time of its discovery, it has been known that the activity of alliinase declines rapidly at neutral pH. Stoll, the discoverer of alliinase, reported the complete loss of the activity of purified alliinase in 14 days at 3 °C (*17, 18*). Under simulated gastrointestinal conditions (37 °C, pH 6.8), Jansen et al. (*4*) reported a 40% loss of activity for pure alliinase in the first hour and a 95% loss if digestive enzymes were present. Our own studies on the stability of alliinase activity in rehydrated freeze-dried garlic powder, both with and without digestive enzymes (not shown), have closely confirmed the results of Jansen et al. (*4*).

As an explanation of what may be taking place during tablet dissolution, it seems likely that the immobile alliinase in the tablets is being activated (upon wetting) before it comes in contact with alliin. This occurs because of permeation of the buffer a short distance into the tablets before the outer surface disintegrates to allow contact between alliin and alliinase, which are located in different cell types (*11, 19*). The slower the disintegration time, the greater would be the amount of time the activated alliinase has to become deactivated before coming in contact with alliin. Hence, slow tablet disintegration would result in greatly reduced alliinase activity and low allicin formation.

When tablets are made with an effective enteric coating, have high tablet alliinase activity, and disintegrate rapidly in the buffer stage, they can release the claimed amount of allicin potential and hence meet the USP 724A dissolution requirements. So far, only one brand has met all three of these conditions, but at least this brand shows that it is very possible for supplements to have high allicin release values that meet the USP guidelines. More importantly, the results have shown that tablets with high dissolution allicin release also show high allicin release in vivo, and those with low dissolution release show low allicin release in vivo.

It has long been proposed that the body may contain sufficient endogenous alliinase activity, particularly in

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the intestinal microorganisms, to be able produce allicin from alliin without alliinase activity from garlic (*18*). Indeed, alliinase-like activity has been reported for *Bacillus subtilis* (*20*), *Escherichia coli* (*21*), and *Pseudomonas cruciviae* (*22*). However, the results from Figure 1 demonstrate that the body possesses little if any alliinase activity because tablets with high allicin potential (high alliin content) and low alliinase activity (low allicin release) produced very low amounts of the allicin metabolite, allyl methyl sulfide. Furthermore, we have found (not shown) that microwave heating of garlic cloves (which inactivates alliinase without decreasing alliin) completely eliminates the presence of allyl methyl sulfide in the breath that is found after consuming raw garlic.

An important question is the minimum level of tablet alliinase activity needed for tablets to achieve high levels of allicin release under USP 724A. An approximate value can be estimated from Tables 2 and 3 by comparing the alliinase activity of the tablet brands that disintegrated in 0.75−1.2 h (brands B, C, I, J, K, S, X) to the 0.75 h allicin release values (% AP) and multiplying by the correction factor needed to give 100% AP. This results in an activity value of about 4000 μg allicin min^{-1} g^{-1} garlic powder of crushed tablets. This activity level was met by five of the brands listed in Table 3. It should be kept in mind that this is the minimum activity value for the crushed tablets, not for the garlic powder used to make the tablets. The activity requirement for the garlic powder may need to be considerably higher, depending on the influence of excipients and conditions used in making the tablets.

The standardization of garlic powder supplements upon allicin potential began about 10 years ago and has been based on the amount of allicin formed when the garlic powder used in the products or the crushed tablets are added to water. Several terms have been used to describe the allicin potential on labels, including allicin yield, allicin potential, total allicin potential, and allicin release (the term proposed by the USP Convention (*7*) is potential allicin). On the basis of the results of the present study, as well as on the failure of garlic tablets with high allicin potential (but low dissolution allicin release) to lower serum cholesterol in five recent clinical trials (*8*) it is now clear that allicin potential should no longer be used for standardization of garlic supplements. *Standardization of any supplement making allicin claims or fresh garlic claims should be based on dissolution allicin release, as determined by USP method 724A*. However, both allicin potential and alliinase activity should be used to monitor the quality of the garlic powders used to make the supplements. Standardization upon alliin content alone (brand U) is only appropriate for products intending to represent cooked garlic, where alliinase has been intentionally inhibited (no such product is currently known, although at least one brand has existed in the past). Standardization of garlic supplements on dissolution allicin release will result in products that closely represent consuming fresh garlic and its associated health benefits. Certainly, no clinical trial should be conducted with a garlic powder supplement that is not standardized upon dissolution allicin release for an effect of garlic that may be related to allicin, as most are.

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