

Utilization of Pectinase Cocktail and High Hydrostatic Pressure for the Production of Aged Black Garlic Juice with Improved Nutritional Value

Jun Ho Kim^{1*}, Jin Woo Kim^{2*}, Su Hyun Yu², Jonghwa Lee¹, Hyung Taek Cho², Wan Heo², Sung Jin Park², Jin Hyup Lee², and Young Jun Kim²

¹Department of Food Science and Biotechnology, Andong National University, Gyeongbuk 36729, Korea

²Department of Food and Biotechnology, Korea University, Sejong 30019, Korea

ABSTRACT: In comparison with raw garlic, aged black garlic has been shown to display multiple pharmacological activities. We recently reported that pretreatment of pectinase cocktail with high hydrostatic pressure (HHP) before the process of aging garlic juice improves its antidiabetic activity and increases *S*-allylcysteine (SAC) content. Thus, this study was designed to investigate the influence of pectinase cocktail with HHP on the quality of aged black garlic juice formation and to identify the optimal manufacturing conditions. In the pretreatment step, garlic juice is heated at 55°C for 24 h. The contents of SAC and total polyphenols were increased with treatment of pectinase cocktail; this increase was greater under HHP processing. In contrast, the total flavonoid content was decreased in all pretreatment conditions. Garlic juice pretreated with pectinase cocktail and HHP had a significantly higher content of SAC in the early phase of aging than raw garlic juice, and the SAC was increased over time in both treatment groups. The total polyphenol content of garlic juice was significantly higher in the pretreatment group during the aging period, and the antioxidant activity of garlic juice showed a positive correlation with polyphenol content. Interestingly, HHP increased the enzymatic activity of the pectinase cocktail.

Keywords: aged black garlic, pectinase cocktail, high hydrostatic pressure, *S*-allylcysteine

INTRODUCTION

Aged black garlic (ABG) is a type of processed garlic that is generally prepared by aging raw garlic at high temperature for 1~3 months. During the aging process, non-enzymatic browning reactions, and enzymatic hydrolysis induce a variety of physicochemical changes to the raw garlic, including of color, texture, taste, flavor, and nutrient content (Ryu and Kang, 2017; Yuan et al., 2016). It has been reported that unstable and unpleasant compounds in raw garlic are converted into stable and tasteless compounds by heat treatment (Zhang et al., 2016). In addition, ABG extracts have been shown to exhibit multiple biological activities both *in vitro* and *in vivo*; specifically the extracts have more potent antioxidant and immunomodulatory properties than raw garlic extracts (Purev et al., 2012; Ryu and Kang, 2017). The improved biological activities of ABG may be associated with increased for-

mation of antioxidants, including polyphenols and flavonoids (Choi et al., 2014; Lee et al., 2009). More importantly, *S*-allylcysteine (SAC), a sulfur-containing amino acid produced by enzymatic hydrolysis during raw garlic aging, has been considered a key compound responsible for the pharmacological activities of ABG (Colín-González et al., 2012). Along with the strong evidence for SAC bioactivity, clinical pharmacokinetic studies have shown that SAC is rapidly absorbed and very stable in the blood after consumption of ABG extracts (Kodera et al., 2002).

Owing to these beneficial changes in compounds during the raw garlic aging process, studies have been conducted to investigate the characteristics of ABG quality under different aging conditions (Bae et al., 2014; Choi et al., 2014; Toledano-Medina et al., 2016; Zhang et al., 2016). These reports have consistently shown that browning intensity, total polyphenol content, and antioxidant activity of garlic are increased with increased aging time

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Correspondence to Young Jun Kim, Tel: +82-44-860-1435, E-mail: yk46@korea.ac.kr

*These authors equally contributed to this work.

Author information: Jun Ho Kim (professor), Jin Woo Kim (Researcher), Su Hyun Yu (Researcher), Jonghwa Lee (Professor), Hyung Taek Cho (Researcher), Wan Heo (Researcher), Jin Hyup Lee (Professor), Young Jun Kim (Professor)

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and/or temperature, whereas moisture content and pH are decreased. Among these studies, only one reported changes in the SAC content of garlic in relation to aging time and temperature: SAC content gradually increased throughout the aging process, but was significantly higher when the garlic was exposed to lower temperatures (Bae et al., 2014). Taken together, these findings suggest that the conventional aging process, which occur at high temperatures for up to 3 months, do not produce the best quality ABG, and more efficient manufacturing process are needed to produce ABG with improved nutritional value.

Recently, we reported that pretreatment of pectinase cocktail with high hydrostatic pressure (HHP) increased the SAC content of ABG juice (ABGJ) with enhanced antidiabetic effects (Kim et al., 2017). The HHP process has been shown to contribute to stabilization of pectinase cocktails and influence enzymatic and nonenzymatic browning reactions during food processing and storage (Heberle et al., 2003; Ioannou and Ghoul, 2013; Tomlin et al., 2013). Thus, to develop optimal manufacturing conditions for ABGJ with improved nutritional value, we investigated changes in quality formation including SAC, polyphenols, and antioxidant capacity during the aging process after pectinase treatment with or without HHP. In addition, we observed the influence of HHP on the enzymatic activity of the pectinase cocktail used in this study.

MATERIALS AND METHODS

Sample preparation

ABGJ was manufactured as previously described (Kim et al., 2017) with some modifications. Fresh peeled garlic (Uiseong, Korea) was ground using a laboratory blender (Waring Commercial, New Hartford, CT, USA), and 3% (v/v) commercial pectinase cocktail (Multifect Pectinase FE, Genencor, Palo Alto, CA, USA), containing pectinase, cellulase, and hemicellulose, was added into the mashed garlic juice. During pretreatment, the pectinase-added mashed garlic juice was incubated with or without HHP (50 or 100 MPa) at 55°C for 24 h using a high-pressure liquefying extractor (DFS-2L, Toyo Koatsu Co., Ltd., Hiroshima, Japan). For aging, raw garlic juice and pectinase +HHP-treated garlic juice were incubated in a thermostatic chamber (TH-G-800, Jeio-Tech, Seoul, Korea) at 30°C with 70% relative humidity for 35 days. The aging temperature used in this study was established based on the previous report that showed higher SAC contents are derived at lower temperatures (Bae et al., 2014). During the 35 days, all garlic samples were collected weekly, filtered, freeze-dried, and then stored at -20°C until future use.

Analysis of SAC

SAC content of garlic juice was determined according to the method described previously (Kubec and Dadáková, 2008) with some modifications. The freeze-dried garlic powder was diluted with distilled water, and 0.1 mL of sample was mixed with 0.25 mL of 10 mM dansyl chloride in acetonitrile and 0.65 mL of 20 mM borate buffer (pH 9.2). The mixture was incubated at room temperature for 15 min, filtered, and subjected to a high-performance liquid chromatography system equipped with a diode-array detector (Nanospace SI-2, Shiseido, Tokyo, Japan) for SAC analysis. The analyte was separated using a Capcell Pak C18 UG120 column (250 mm×4.6 mm ID, 5 µm, Shiseido). The solvent system consisted of two eluents: eluent A was 50 mM sodium acetate in distilled water (pH 5.0 adjusted by formic acid) and eluent B was methanol. A gradient elution was employed at different ratios of A : B (v/v); 7:3 (0 min), 6:4 (35 min), 2.5:7.5 (70 min), and 7:3 (95 min).

Determination of total polyphenols, total flavonoids, and antioxidant capacity

The total polyphenol content was determined using Folin-Ciocalteu's colorimetric method with some modifications as described in a previous report (Kim et al., 2010) and was expressed as mg of gallic acid equivalents (GAE). Total flavonoid content was measured using aluminum chloride colorimetric assays as previously reported (Kamtekar et al., 2014) and expressed as mg of rutin equivalents (RE).

The free radical scavenging activity of garlic juice was measured using the 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) and 1,1-diphenyl-2-picrylhydrazil (DPPH) assays. For ABTS assays, 2,2'-azobis dihydrochloride (1 mM) and ABTS (2.5 mM) were dissolved in phosphate-buffered saline (100 mM), heated at 70°C for 30 min, and then cooled to room temperature. The diluted ABTS solution (0.98 mL) was then mixed with sample solution (0.02 mL), and the absorbance was measured at 734 nm after 20 min using a spectrophotometer (UV 2100, Shimadzu Co., Kyoto, Japan). For DPPH assays, 1 mM of DPPH was dissolved in ethanol, and 1.95 mL of DPPH solution was mixed with 50 µL of sample solution. The mixture was incubated at room temperature for 30 min, and then the absorbance was measured at 517 nm. The ABTS and DPPH radical scavenging activities were expressed as vitamin C equivalents.

Measurement of enzyme activity

The enzymatic activity of the pectinase cocktail was determined by the rate of release of reducing ends from polygalacturonate, according to methods described previously (Li et al., 2015; Reid and Ricard, 2000) with some modifications. Briefly, two test tubes containing 2 mL of

diluted enzyme stock were equilibrated to 55°C and 10 mL of substrate stock solution was added to each tube. Tubes with or without HHP treatment (50 MPa) were incubated in a water bath at 55°C for 0.5, 1, 2, 3, or 4 h. Solution A was prepared by sequentially dissolving Na₂CO₃, glycine, and CuSO₄ in distilled water and was used as a time 0 control. Neocuproine HCl was then added to each tube, and the tubes were mixed and incubated at 100°C for 12 min. Finally, the tubes were cooled and diluted, and the absorbance was recorded at 450 nm. Enzyme activity (U/mL) was calculated as follows:

$$\text{Activity} = \frac{\mu\text{g galacturonic acid released}}{0.2 \times 194.1 \times t}$$

where 0.2 is the enzyme broth volume used in the assay, 194.1 is the molecular weight of galacturonic acid, and t is the reaction time in min. One unit (U) is equivalent to 1 μmol product released per min.

Statistical analysis

Data were analyzed by one-way ANOVA using the SAS software for Windows release 9.2 (SAS Institute Inc., Cary, NC, USA). One-way ANOVA with repeated measures was performed to assess mean differences between groups. The least squares means option using a Tukey-Kramer adjustment was used for multiple comparisons

between the experimental groups. Data are shown as mean \pm standard error (SE). P -values <0.05 are reported as statistically significant.

RESULTS AND DISCUSSION

Effects of pectinase pretreatment with HHP on quality formation of ABGJ

To investigate the influence of pectinase pretreatment with HHP on quality formation of ABGJ, we examined changes in the contents of SAC, polyphenols, and flavonoids, and antioxidant capacity before and after the aging process. In the pretreatment step, incubation at 55°C for 24 h with or without the pectinase cocktail significantly increased the SAC content of garlic juice (Fig. 1A). After incubation with pectinase, the total polyphenol content was increased but this increase was not significant (Fig. 1B). Importantly, the contents of SAC and polyphenols were highest when garlic juice was subjected to cotreatment of pectinase with HHP at 50 or 100 MPa. In contrast, the total flavonoid content of garlic juice was decreased during the heating process; this change was greatest when heating with pectinase (Fig. 1C). The increase in phenolics and decrease in flavonoids heating are consistent with a previous study using onion, which was attributed to the hydrolysis of C-glycoside bonds of the

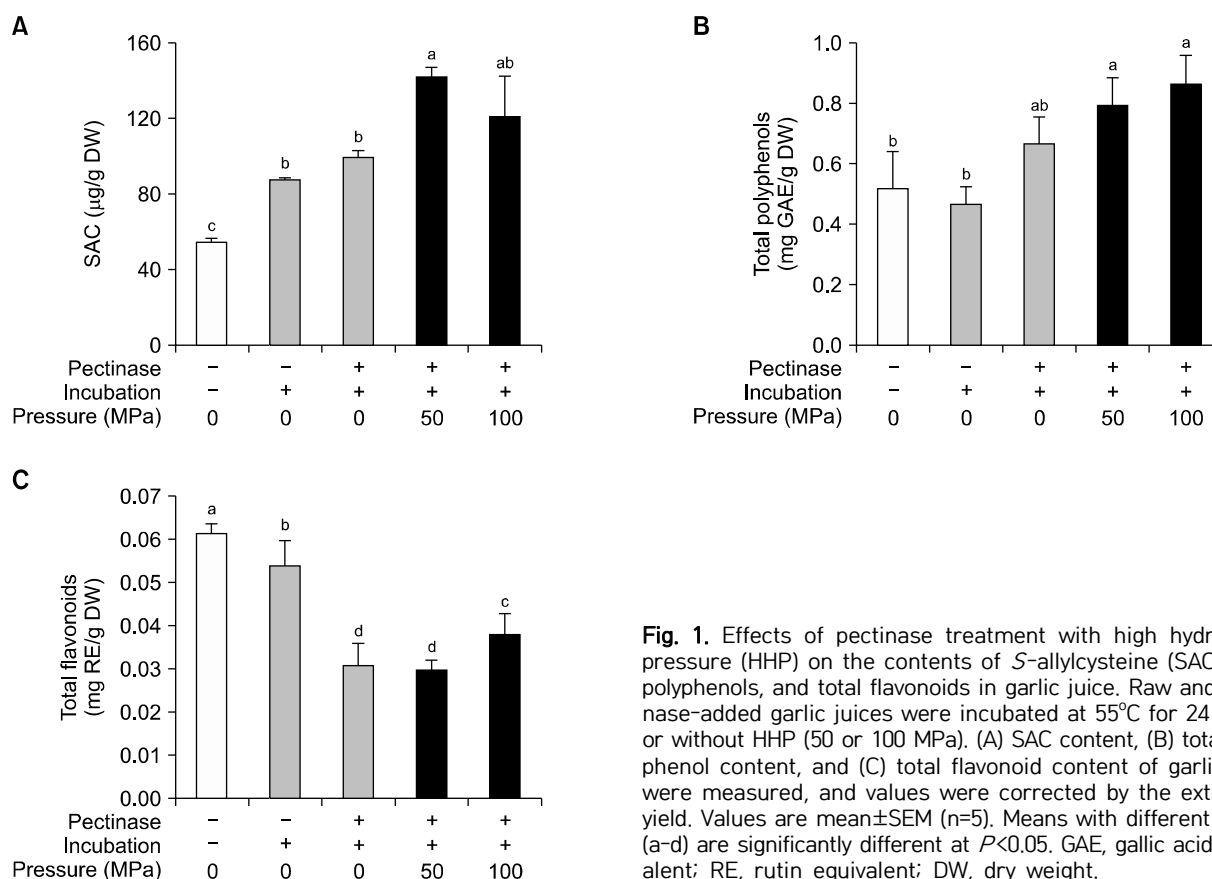


Fig. 1. Effects of pectinase treatment with high hydrostatic pressure (HHP) on the contents of *S*-allylcysteine (SAC), total polyphenols, and total flavonoids in garlic juice. Raw and pectinase-added garlic juices were incubated at 55°C for 24 h with or without HHP (50 or 100 MPa). (A) SAC content, (B) total polyphenol content, and (C) total flavonoid content of garlic juice were measured, and values were corrected by the extraction yield. Values are mean \pm SEM ($n=5$). Means with different letters (a-d) are significantly different at $P<0.05$. GAE, gallic acid equivalent; RE, rutin equivalent; DW, dry weight.

flavonoids (Sharma et al., 2015).

Since the SAC content of garlic juice was increased by HHP at 50 MPa and slightly decreased at 100 MPa (Fig. 1A), we used garlic samples prepared at 50 MPa for further comparisons with raw garlic juice. As expected, the SAC content of garlic juice increased progressively over time throughout the aging process at 30°C, although that of raw garlic juice was slightly decreased after 28 days (Fig. 2A). In a previous study using different aging temperatures, garlic bulbs heated at low temperatures had higher amounts of SAC than those heated at higher temperatures: the SAC content increased up to 124.67 µg/g when heated at 40°C for 45 days (Bae et al., 2014). Of note, our current results show that the SAC content of raw garlic juice increased to 177.70 µg/g when heated at 30°C for 28 days. Importantly, garlic juice pretreated with pectinase under HHP showed 121.74 µg/g of SAC before aging, and the SAC content increased to 181.42 µg/g after aging at 30°C for 35 days. These results suggest that co-treatment of pectinase with HHP before heating at 30°C was more efficient for producing ABGJ with enhanced SAC content than the conventional aging process at higher temperatures.

A significant increase in total polyphenols in garlic juice was observed during aging when following pretreatment with pectinase and HHP (Fig. 2B). In addition, results from ABTS and DPPH radical scavenging activity assays showed that garlic juice had significantly higher antioxidant activity when pretreated with pectinase and HHP at different times during the aging process (Fig. 2C and D). Regardless of pretreatment, the polyphenol content and antioxidant activity showed a similar pattern of change, whereby they increased until day 21 of aging and then decreased. This positive correlation between polyphenol content and antioxidant activity was also observed in a previous study using a whole bulb of garlic; both increased to day 24, and then decreased during the aging process at 72°C (Toledano-Medina et al., 2016). Although SAC has been reported as a potent antioxidant compound (Dvořáková et al., 2016; Pérez-Severiano et al., 2004), our current data, combined with previous findings, indicate that polyphenols could be the major determinant of the radical scavenging potential of ABGJ.

The use of HHP as a nonthermal process for food preservation has been shown to inactivate microorganisms as well as enzymes responsible for shortening the shelf-life

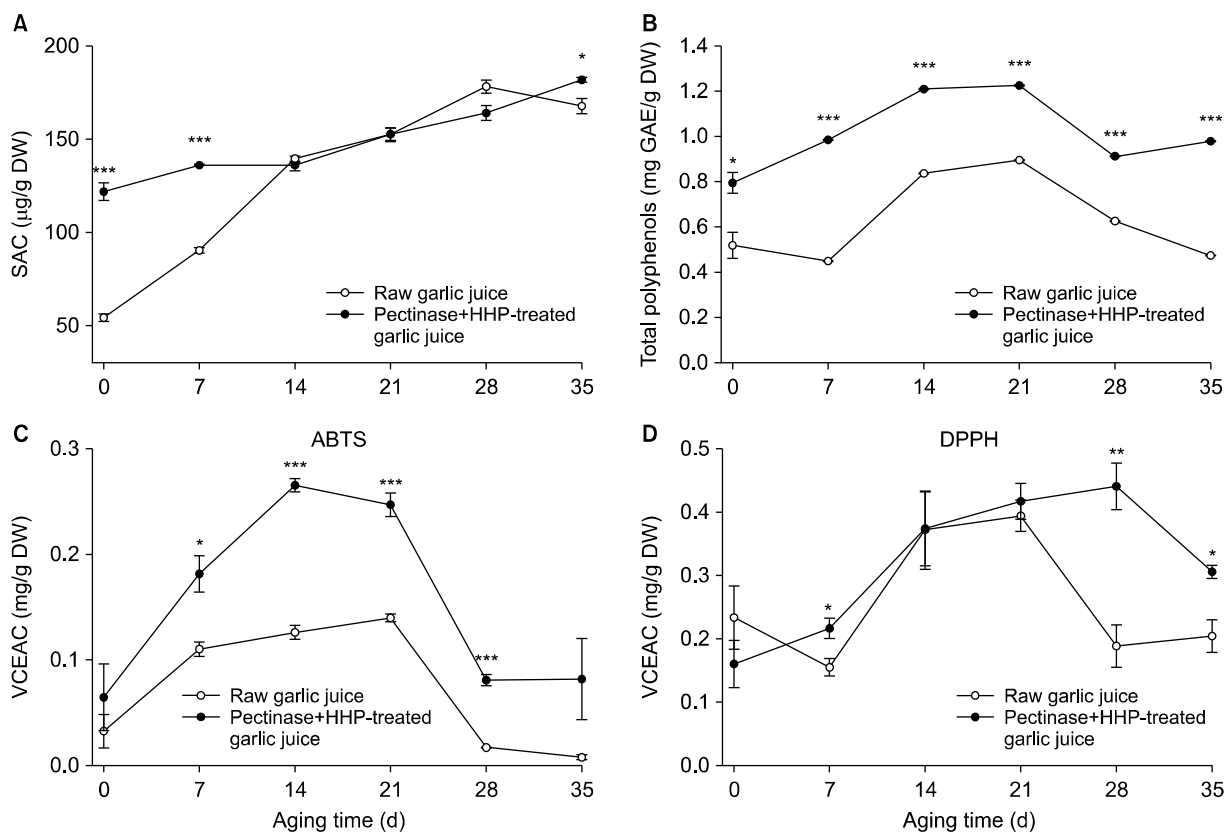


Fig. 2. Effects of pectinase treatment with high hydrostatic pressure (HHP) on the *S*-allylcysteine (SAC) and total polyphenol contents, and antioxidant capacity of garlic juice during the aging process. Raw and pectinase-treated garlic juice with HHP (50 MPa) were subjected to the aging process at 30°C for 35 days. (A) SAC content, (B) total polyphenol content, (C) 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical scavenging activity, and (D) 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging activity of garlic juice were measured, and values were corrected by the extraction yield. Values are mean±SEM (n=5). * $P<0.05$, ** $P<0.01$, and *** $P<0.001$ vs. raw garlic juice. GAE, gallic acid equivalent; VCEAC, vitamin C equivalent antioxidant capacity; DW, dry weight.

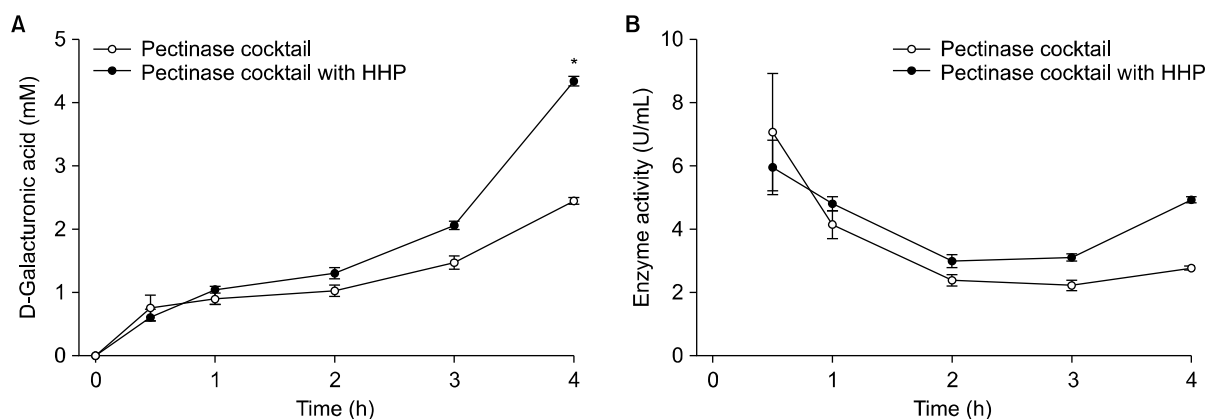


Fig. 3. Influence of high hydrostatic pressure (HHP) on the enzymatic activity of pectinase cocktail. The enzymatic activity of pectinase cocktail was determined by the rate of release of reducing ends from polygalacturonate. (A) Amounts of reducing sugar released and (B) the corresponding enzymatic activity. Values are mean \pm SEM ($n=6$). * $P<0.05$ vs. control.

of food products (Garriga et al., 2004; San Martín et al., 2002). HHP inactivates microbes lethally and/or sub-lethally whilst minimizing degradation of flavor, color, and nutritional components (Yamamoto, 2017). These findings reveal the advantages of HHP technology as a promising nonthermal process for food processing. Therefore, further studies to evaluate the influences of HHP on microbial safety and the sensory properties of ABG may provide more meaningful data.

Influence of HHP on pectinase enzyme activity

Since pretreatment of pectinase with HHP resulted in significant increases in the contents of SAC and total polyphenols, and antioxidant activity of garlic juice before and after aging, we next determined whether HHP may enhance pectinase enzymatic activity. The content of released galacturonic acid and the corresponding enzymatic activity was higher in the HHP-treated group at 50 MPa than in the control group, with significant differences observed at 4 h (Fig. 3). Similarly, HHP treatment at 200~400 MPa delayed the rate of pectinase cocktail inactivation when treated at 77°C (Tomlin et al., 2013). These findings suggest that HHP could be effectively used to stabilize the commercial pectinase cocktail at a high temperature. Since pectinase cocktails are widely used in juice processing to improve extractability, clarity, and functional properties (Puri et al., 2012), our current findings provide useful information that could be applied to the juice industry, including to garlic juice processing. However, the question of how HHP stabilizes and increases pectinase activity remains unanswered, and further studies to investigate changes to enzyme structures with HHP are needed.

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AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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