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Review

Black Garlic: Processing, Composition Change, and Bioactivity

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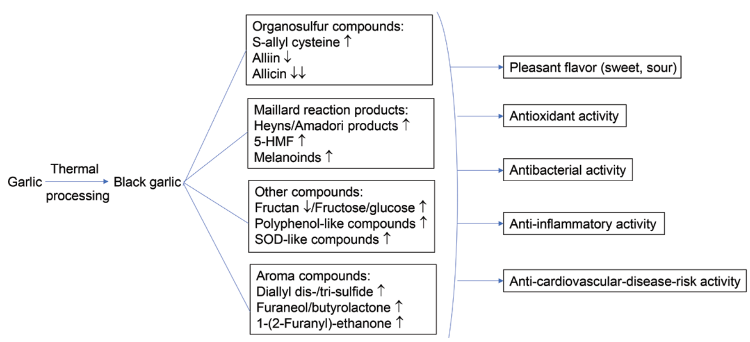
ABSTRACT

Garlic (*Allium sativum* L.) has been not only consumed as flavor vegetable, but also recorded as a prophylactic and therapeutic medicinal plant since ancient time. Its characteristic pungent flavor limited its application in food product development. Black garlic made from garlic by a thermal processing under certain temperature and relative humidity tastes slightly sweet without pungent flavor, and has extended shelf-life. The present work presented recent development in black garlic processing scheme, composition changes during thermal processing of black garlic, especially formation of Maillard reaction products, and some bioactive properties of black garlic.

GRAPHICAL ABSTRACT



1. INTRODUCTION

Center of origin of garlic (*Allium sativum* L.) is unclear and still in debate. Central Asia was the primary center of garlic domestication and cultivation. The wild type of *Allium longicuspis* Regel has been found in the Thien Shain Mountains of Central Asia, and been proposed as the progenitor of garlic, since both species have a similar morphology [1–3]. Garlic has been recorded as a prophylactic as well as therapeutic medicinal plant since ancient time. Many favorable effects of garlic and its preparations include antioxidant effect, antimicrobial effect, reduction of cancer risk, reduction of risk factors for cardiovascular diseases [4].

Garlic is rich in inulin [inulin-type fructan of β-(2,1)linkage]. Starch and inulin are main storage polysaccharides in plant foods. Inulin is the second most abundant plant storage polysaccharide after starch, and is found in garlic, leeks, bananas, chicory, and Jerusalem artichokes [5]. Garlic contains inulin 23% on a fresh garlic basis or >75% (w/w) on a dry garlic basis. The inulin isolated from garlic is a series of homologous oligo- and poly-saccharides, with molecular weights of 1000–10,000 Da and Degree of Polymerization (DP) up to 58 [6].

There are two major sources of plant foods containing organosulfur compounds, Allium vegetables, such as garlic (*A. sativum*), onion (*Allium cepa*), and leek (*Allium ampeloprasum* var. porrum); and cruciferous vegetables, such as broccoli, cabbage, and cauliflower. Among them, garlic is the most consumed and studied vegetable, mainly due to its organosulfur compounds [7].

Garlic bulbs are usually stored for several months after harvest to ensure year-round supplies for customers. The proper storage conditions are important to extend shelf life and prevent spoilage, such as surface discoloration, withered, sprouting, and moldy. Commercially, garlic bulbs were stored at 0–3°C. It has been reported that the optimum temperature for induction of sprouting and moldy is about 4°C. The sprouting and moldy were suppressed

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when garlic bulbs were stored at ~2°C; the firmness and taste were retained at ~3°C for 9 months [8].

Bulbs of black garlic were discovered in the cargo of a medieval shipwreck during the exploration of the 15th-century Copper Wreck, which sank near the port of Gdańsk (southern Baltic Sea, N Poland) [2]. The black garlic existed about 500 year ago, and was well preserved. It revealed that black garlic has extended shelf-life. Garlic could be transformed to black garlic under a thermal processing with decreased water activity and increased Maillard reaction products [9], which could be contributed to extended shelf-life of black garlic.

2. BLACK GARLIC PREPARATION

Black garlic was thermal processed from fresh garlic under controlled temperature and humidity without using additives, which was ready-to-eat with sweet taste and little pungent odor and taste. It appeared first in 1999, and produced by Japanese Kamimura in Mie prefecture, Japan, who also filed manufacturing patent of the black garlic in Japan Patent Office [10].

Black garlic is generally processed at the temperature range from 40 to 90°C, the Relative Humidity (RH) of 60–90%, and incubation period of 10–90 days. There are various thermal processing plans. For example, 70°C with 90% RH for up to 35 days; 70 or 85°C with 70% RH for up to 45 days; a four-step continuous process (11 days): 90°C in oven for 2 days → 80°C for 4 days → 60°C for 4 days → 40°C for 1 day; a five-step continuous process (14 days): 90°C and 100% RH for 34 h → 60°C and 60% RH for 6 h → 75°C and 70% RH for 48 h → 70°C and 60% RH for 60 h → 65°C and 50% RH for 192 h [11].

Under 80% relative humidity and 3-day oven-drying at 60°C, the color change of garlic was very slow, and could not completely be black; at 70°C, the color change is much faster than that at 60°C; at 70–80°C, black color is homogeneous; at 90°C, bitter and sour tastes would appear. The appearance of black garlic at 70°C was dry with better elasticity and quality; at 80°C, bone-dry with burning; at 90°C, very hard with an obvious burning smell. When the moisture content reaches the range of 400–500 g/kg, BG has optimal softness and elasticity; 350–400 g/kg, BG would be much drier with poor elasticity; below 350 g/kg, BG would be very dry and hard [12]. We have developed a novel process of black garlic by soaking garlic cloves in Maillard reaction solution and heated for up to 90°C for only 3 days [13].

Black garlic has also been called aged garlic, which is produced at controlled temperature and humidity. Aged Garlic Extract (AGE) is made differently, and is prepared by soaking sliced garlic cloves in an aqueous ethanol solution (i.e., 15–20% ethanol), and then extracted/aged up to 1.5 years (10–18 months) at room temperature in an air-tight container [4,14].

3. COMPOSITION CHANGES

Major components of garlic are carbohydrates (36%; including 1% sugars and 2% dietary fiber), protein (6%), and fat (0.5%) [15]. In other report, garlic contained 60.3% water, 28.7% carbohydrates, 8.4% protein, and 0.1% fat [11]. The carbohydrates include fructan (23.2–27.8 g/100 g), sucrose (0.6–0.7 g/100 g), fructose (0.1 g/100 g), glucose (0.04–0.05 g/100 g fresh garlic) [16]. Folin–Ciocalteu's and Ellman's methods were used to quantitation of polyphenols and thiols in the crushed garlic solution. Their concentrations were determined as 9.5 ± 0.5 and 3.4 ± 0.4 μmol/g, respectively [17].

In comparison of garlic and black garlic, garlic weight was decreased 64%, soluble solid content (°Brix) increased 13%, water activity (aw) changed slightly from 0.97 to 0.93, when garlic was manufactured at 60°C and 90% RH for 45 days. In comparison of garlic and black garlic, moisture content was decreased one to twofold [11].

Carbohydrates were increased one to twofold, sucrose was increased 1.3–1.6 folds, fructose was increased sixfold to 108 folds, and glucose was increased twofold to 13 folds in black garlic in comparison with fresh garlic [11]. The carbohydrate content is increased 28.7–47.0% when garlic was manufactured at 60°C and 90% RH for 45 days. Fructan was decreased ~6 folds when garlic was incubated at 55°C with 80% humidity for 90 days [18]. Monosaccharides became dominant saccharides in black garlic, with small amounts of polysaccharides and disaccharides [19]. Most saccharides and associated derivatives were found in black garlic (60–80°C, 36 days), and a few found in garlic [20].

The amino acids are increased 2.5 times when garlic was manufactured at 60°C and 90% RH for 45 days. Black garlic were prepared at the temperatures of 60–80°C for 36 days. Many amino acids and metabolites were identified in both garlic and black garlic, although their amino acid profiles were different [20].

The polyphenol content (g GAE (gallic acid equivalents)/kg) increased 2.8%, and antioxidant activity (TROLOX equivalents/kg) increased 6.7%, when garlic was manufactured at 60°C and 90% RH for 45 days. In comparison of garlic and black garlic, phenol-like compounds were increased three to fourfold, flavonoid-like compounds were increased 1.5 to eightfold, antioxidant compounds were increased 1.6–12 folds [11]. In general, black garlic contains the polyphenol content that is three times higher in whole black garlic bulbs and six times higher in peeled black garlic cloves in comparison with fresh ones of garlic.

The allicin was decreased to trace level, and *S*-allyl cysteine was increased four to eightfold in black garlic in comparison with fresh garlic [11]. The amino acids and the *S*-allyl-L-cysteine contents are increased 2.5 and eight times, respectively, when garlic was manufactured at 60°C and 90% RH for 45 days. *S*-allyl cysteine content was relatively high in black garlic [15]. *S*-allyl cysteine is biosynthesized via the elimination of glutamic acid from γ-glutamyl-*S*-allyl cysteine by γ-glutamyl transpeptidase. The sulfur oxidation of *S*-allyl cysteine by flavin-containing monooxygenase forms *S*-Allyl-L-Cysteine Sulfoxide (ACSO). ACSO can be converted to allylsulfenic acid (2-propene-1-sulfenic acid) and aminoacrylic acid by alliinase. Aminoacrylic acid is then spontaneously converted to pyruvic acid and ammonia. Two molecules of allylsulfenic acid produce allicin (diallyl thiosulfinate). ACSO is the precursor of thiosulfinate compounds of Allium vegetables [7,21].

Relatively high concentrations of water-soluble organosulfur compounds were found, such as γ-glutamyl-*S*-allyl-L-cysteines and *S*-allyl-L-cysteine sulfoxides (alliin) in intact garlic, and *S*-allyl cysteine in black garlic [15]. Black garlic were prepared at the temperatures of 60–80°C for 36 days, and its methanol–water (50:50, v/v) extract was analyzed by liquid chromatography coupled to quadrupole time of flight (LC–QTOF) MS/MS. Major changes of molecular features in garlic occurred during 2–6 days. Garlic had most organosulfur compounds, especially cysteine derivatives; while disulfides, two sulfoxide derivatives and a sulfothionate appeared only in black garlic. Among organosulfur compounds, the contents of γ-glutamyl-S-alk(en)yl-L-cysteine family and sulfoxides group (alliin, cycloalliin and methiin) were decreased gradually; in thiosulfinate group, allicin and thiacremonone were decreased drastically while diallyl disulfide and diphenyl disulphide were increased drastically after 6 days [20].

4. FORMATION OF MAILLARD REACTION PRODUCTS

The Maillard reaction, also the amino-carbonyl reaction, is known as a non-enzymatic reaction between reducing sugars and the amino group in amino acids, peptides and proteins. This reaction occurs during food processing and storage, and yields browning and characteristic flavor compounds to food products [22].

The Maillard reaction was categorized into three stages, initial stage started with the reaction between carbonyl groups of reducing sugars and amino groups of amino acids and till formed the Amadori product (←glucose) or Heyn's product (←fructose); intermediate stage included sugar fragmentation and amino acid degradation with formation of various intermediates, especially dicarbonyl compounds; late stage had polymerization of the intermediates with formation of high molecular weight products, especially melanoidins [23].

The 2-Furoylmethyl-γ-Aminobutyric Acid (2-FM-GABA), 2-Furoylmethyl-Lysine (2-FM-Lys, furosine), and 2-Furoylmethyl-Arginine (2-FM-Arg) were identified in black garlic, with a descending order of concentrations of 2-FM-Lys > 2-FM-Arg >> 2-FM-GABA. Those furoylmethyl amino acids, especially furosine, are formed after the acid hydrolysis of Amadori and Heyn's products, and have been recognized as indicator compounds in initial stage of Maillard reaction [24].

The 5-Hydroxymethylfurfural (5-HMF) and 2-acetylpyrrole were the intermediate products of Maillard reaction, and were identified in black garlic. 5-HMF has been detected as indicator compound in intermediate stage of Maillard reaction. 2-Acetylpyrrole contributes a pleasant flavor to black garlic [25]. Twenty six compounds including 12 new structures and 14 known compounds were identified in black garlic, and were considered to be produced in Maillard reaction pathways. The new identified compounds include five pyrazine derivatives containing 1,2,3-butanetriol moiety; four pyrrole derivatives containing arginine, aspartic acid, glutamic acid, and carbaldehyde moieties, respectively; three organic acid-type Maillard products containing carboxylic acid, acetic acid, picolinic acid moieties, respectively. Those compounds have been shown to have good antioxidant activity [26]. The high-molecular-weight compounds of 4.07 and 3.98 kDa were fractionated as melanoidins in the late stage products of Maillard reaction, which were increased markedly as thermal processing progressed in black garlic [23].

The aroma compound profile of fresh garlic was relatively simple, with many sulfur-containing compounds and few aldehydes and ketones. There were 20 volatile compounds were identified and classified into eight groups: derivatives from *S*-alk(en)-yl-L-cysteine, other sulfur-containing compounds, alcohols, aldehydes, furans, acids, ketones, and other compounds [27]. Our recent study identified a total of 52 aroma-active compounds in black garlic, which could be classified into seven groups, including alcohols, aldehydes, ketones, sulfur-containing compounds, heterocyclic compounds, organic acids, and other compounds. There were 24 aroma-active compounds with $\log_2$Flavor Dilution (FD) ≥ 2, including eight sulfur-containing compounds, six heterocyclic compounds, two carbonyl compounds, two alcoholic compounds, three acids, and three unknown aroma compounds. The eight sulfur-containing compounds included allyl methyl trisulfide (cooked garlic), 2-vinyl-4*H*-1,3-dithiine (garlic), diallyl disulfide (garlic), diallyl trisulfide (sulfur), 3-vinyl-1,2-dithiacyclohex-4-ene (spicy), diallyl sulfide (garlic), 3*H*-1,2-dithiole (putrid), and dimethyl sulfoxide (sulfur). Among them, diallyl disulfide, 3-vinyl-1,2-dithiacyclohex-4-ene, and diallyl sulfide have also been reported in fresh garlic. The six heterocyclic compound included furaneol (caramel), 2(5*H*)-furanone (burnt), 1-(2-furanyl)-ethanone (balsamic), 2-acetyl-1-pyrroline (roasted nut), 5-heptyldihydro-2(3*H*)-furanone (apricot), and butyrolactone (caramel). The two carbonyl compounds included 3-(methylthio)propionaldehyde (cooked potato) and 1-hydroxy-2-butanone (savory), which were not detected in fresh garlic. The two alcoholic compounds included (*E*,*Z*)-2,6-nonadien-1-ol (cucumber) and allyl alcohol (cooked garlic); three acids included acetic acid (sour), 3-methylbutanoic acid (sweat), and propanoic acid (pungent); and other three were unknown aroma compounds. Among 24 aroma-active compounds, 10 aroma-active compounds were identified as key aroma compounds with Odor Activity Value (OAV) > 1. They were acetic acid, allyl methyl trisulfide, furaneol, diallyl disulfide, diallyl trisulfide, (*E*,*Z*)-2,6-nonadien-1-ol, 3-methylbutanoic acid, 5-heptyldihydro-2(3*H*)-furanone, diallyl sulfide, propanoic acid. Although there were five aroma-active compounds with OAVs less than 1, they were included as the key aroma-active compounds based on omission experiments, including 2(5*H*)-furanone, 1-(2-furanyl) ethanone, 1-hydroxy-2-butanone, dimethyl sulfoxide, and butyrolactone. In other study, allyl mercaptan and 1,3-dithiane were detected only in fresh garlic, while dimethyl trisulfide and allyl alcohol (2-propen-1-ol) was detected only in black garlic. The allyl alcohol (2-propen-1-ol), the most abundant volatile compound in black garlic, contributes to flavor of heated garlic. *S*-alk(en)-yl-L-cysteine, dimethyl disulfide, and allyl methyl disulfide were decreased greatly in black garlic in comparison with that in fresh garlic. Diallyl disulfide was the major sulfur volatile (45%) in fresh garlic, and was low (7%) in black garlic. Di-allyl trisulfide and allyl methyl trisulfide were increased greatly in black garlic in comparison with that in fresh garlic [27].

The key aroma compounds of acetic acid, propanoic acid, furaneol (caramel), 5-heptyldihydro-2(3*H*)-furanone (apricot), 2(5*H*)-furanone (burnt), 1-(2-furanyl)-ethanone (balsamic), 2-acetyl-1-pyrroline (roasted nut), and butyrolactone (caramel), 3-(methylthio)propionaldehyde (cooked potato), are all Maillard reaction products. The 5-heptyldihydro-2(3*H*)-furanone and (*E*,*Z*)-2,6-nonadien-1-ol could be derived from fatty acids, and be inter-reaction products of Maillard reaction and lipid peroxidation [22]. Acetic acid along with furfural and furfural derivative, 2-acetylfuran were also detected in black garlic [27].

Table 1 | Biological effects of black garlic

Models/human studies	Black garlic doses	Effects	References
P. aeruginosa	4% (MIC)	Biofilm ↓	[29]
S. aureus	3.1 μg/mL (MIC)	Growth ↓	[28]
L. monocytogenes	12.5 μg/mL (MIC)	Growth ↓	[28]
HL-60 cells	2 mg/mL	Growth ↓	[9]
RAW264.7 cells	250 μg/mL	NO ↓, TNF-α ↓	[11]
Drosophila	4 mg/mL	Healthspan ↑	[9]
Mouse	1 mg × three times injection	Tumor size ↓	[10]
Rats	125 mg/kg/12 h × 30 days	Cholesterol ↓ MDA ↓	[31]
Human	2.56 g/day × 45 days	$\gamma\delta$-T cells ↑, NK cells ↑	[30]
Human	2.4 g/day × 12 months	Artery plaque ↓	[32]

5. BIOACTIVITY OF BLACK GARLIC

Bioactivity of black garlic with increasing antioxidant activity and NK cell activity, and decreasing translated tumor size in BALB/c mouse were first reported in a Japanese new paper in 2006 and in a scientific publication in 2007, of which the study was initiated in 2003 and carried out by Japanese researcher Jinichi Sasaki in Faculty of Medicine of Hirosaki University in Aomori Prefecture, Japan [10].

The black garlic at the concentrations of 0.25–4 mg/mL did not induce DNA fragmentation, while a slight fragmentation was observed at the concentration of 0.25 mg/mL of garlic. The cell-growth inhibition of HL-60 tumor cells was observed at an IC_{50} 0.03 mg/mL of raw garlic. While the cell-growth inhibition was not observed at an IC_{50} 0.9 mg/mL, and was observed only at 2 mg/mL of black garlic. Black garlic at 4 mg/mL induced an extension (~9 days) of health span in Drosophila in comparison with raw garlic [9].

Black garlic showed antibacterial activity against the tested clinical isolates, of which the best Minimal Inhibitory Concentration (MIC) results were obtained for the Gram-positive bacteria methicillin-resistant *Staphylococcus aureus*, followed by *Enterococcus faecalis* and *Listeria monocytogenes*. The MIC results for Gram-negative bacteria were best for *Escherichia coli*, followed by *Pseudomonas aeruginosa* [28]. An inhibition percentage of >55% on biofilm-forming *P. aeruginosa* was observed at a concentration of 20% (MIC), and ~35% at 10% (1/2 MIC) of black garlic fermented extracts [29].

Black garlic has been demonstrated to have anti-inflammatory activity, by decreasing LPS-induced production of Nitric Oxide (NO), Tumor Necrosis Factor-α (TNF-α), and prostaglandin-E2 in RAW264.7 cells, TNF-α-induced activation of Nuclear Factor Kappa B and expression of intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 in human umbilical vein endothelial cells and human endometrial stromal cells. Some compounds showing anti-inflammatory effects in AGE were identified as pyruvate, 2-linoleoylglycerol, and 5-hydroxymethylfurfural [11].

A study recruited 120 healthy individuals, and divided them into placebo-control group (60) and AGE-intervention group (60), who consumed AGE 2.56 g/day for 45 days. The lymphocytes of $\gamma\delta$-T cell and NK cell were able to proliferate better in *ex vivo* cultures than cells from those who consumed the placebo. The $\gamma\delta$-T cells, existed numerously in the epithelial linings of intestine, lung and urinary tract, nearly doubled their ability to proliferate, whereas the NK cells almost tripled their proliferation numbers when compared with control values. The expression of CD314 (natural killer group 2 member D), a marker of activity, on the surface of NK cells was significantly greater after AGE consumption [30].

Effects of AGE on cardiovascular functioning in a rat model of Metabolic Syndrome (MS) were investigated. The AGE decreased cholesterol and Malondialdehyde (MDA) levels; increased glutathione level and glutathione peroxidase activity; increased vascular relaxation and decreased vascular contraction [31]. The AGE (2400 mg/day for 12 months) exhibited a regression in coronary artery plaque, indicated by Low-Attenuation Plaque (LAP) volume. There was 29% reduction in LAP volumes in the AGE group as opposed to 57% progressed LAP in the placebo group of patients with type 2 diabetes mellitus [32]. Those biological effects of black garlic in recent reports have been summarized in Table 1.

CONFLICTS OF INTEREST

The author declares no conflicts of interest.

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