



Review Article

Psychochemical changes and functional properties of organosulfur and polysaccharide compounds of black garlic (*Allium sativum* L.)

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ABSTRACT

Background: Black garlic is one of the functional food products made from garlic which is processed through aging to improve sensory value and nutritional quality. Aging conditions has a major impact on the psychochemical and functional properties changes of black garlic which is closely related to organosulfur compounds and polysaccharides as the largest component in garlic.

Scope and approach: The method used in this research is a systematic review with the aim of research to determine the relationship between reactions during aging and changes in organosulfur, polysaccharides and non-enzymatic browning product compounds as well as the function of black garlic by focusing on certain aspects of aging including temperature, humidity, time, microorganism activity, and pre-treatment application.

Key findings and conclusions: Maillard reaction and polysaccharide degradation are still be the dominant reactions and play an important role in black garlic production. High hydrostatic pressure pretreatment could maintains the quality of black garlic so that the black garlic has the same taste characteristics as black garlic in general. Antioxidant properties in black garlic shown increase during thermal treatment. In addition, it is known that the activity of microorganisms plays a role and being potential to increase the quality value of black garlic as well as the antimicrobial activity.

1. Introduction

Garlic is a part of *Allium* family which certified generally recognized as safe (GRAS) that has a variety of health benefits. Their biological activity makes garlic become widely applied in various sectors both as food additives and in mixtures of herbal medicines (Ezeorba et al., 2022). Black garlic or might be called aged garlic is a processed through aging methods without an addition of any additional substances in a high and controlled temperature (40–60 °C) and humidity (60–90%) (Jing, 2020; Kimura et al., 2017; Xiong et al., 2018) (see Fig. 1).

Among the variety of garlic nutrients, the organosulfur is one of the dominant compounds that greatly affects organoleptic quality and functional characteristics of garlic (Farías-campomanes et al., 2014). Allicin is the major thiosulfinate component in garlic, which is as much as 70–80% of the amount of organosulfur. These compound and other thiosulfates responsible in producing a distinctive smell, taste, and functional properties (especially antibacterial) in garlic (Prati et al., 2014). However, this unique smell and pungent taste on fresh garlic

make a discomfort thing and possibly causing gastrointestinal hurt in some people (Ryu and Kang, 2017). Therefore, scientists mostly use heating principle particularly aging to eliminate the stinging characteristics of garlic. Aging process can also stimulate phytochemicals change and increasing functionality properties of black garlic (Banan et al., 2019). A high-temperature condition turns garlic appearance into black, soft, odorless, and has a sweet-sour taste. This condition also extended black garlic's shelf-life, longer than garlic (Setiyoningrum et al., 2021).

The aging process or spontaneous fermentation has a major impact on garlic such as the increase of polysaccharides concentration, total reducing sugars, proteins, phenolic compounds, organosulfur compounds, and melanoidin (Lu et al., 2017). Not only on phytochemical content, aging also affect microbiological activity and functional properties changes during the aging process (Sudjatni, 2020). The three methods of heat treatment, aging and fermentation have been studied to determine their advantages and disadvantages. Heat treatment can result in non-enzymatic browning or Maillard reactions which can cause

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physical and chemical changes. The advantages of this method is Garlic has a chewy texture and increases the soluble solids content, making it taste sweet after the heating process. with the pH decreasing to close to 3.4 and high temperatures which can make the shelf life of black garlic longer (Nencini et al., 2011). But the disadvantages there changes in color, taste, texture and even macronutrients and other compounds.

The aging process can increase the polyphenol content, Advantages: resulting in increased antioxidant activity. During the aging process, there are organosulfur compounds which act as water-soluble bioactive compounds with high antioxidant content. The content is around 20–30 µg/g in raw garlic, and increases up to six times after the aging process (Toledano Medina et al., 2016). The disadvantage of this method is aging process at high temperatures can damage the tissue and cells of black garlic under high temperatures and humidity.

Fermentation of black garlic is a method that involves natural microorganisms found in the substrate with spontaneous reactions. The advantages of this method is microbial fermentation contains a lot of glucose and mannose, and produces lactic acid which is useful in strong antioxidant activity and can improve immunology and inhibit bacteria (Vijayabaskar et al., 2011), there is also the disadvantage which is need a large scale fermentation is needed to provide accurate data on the comparison of fermented and non-fermented black garlic (Qiu et al., 2020). A high temperatures and humidity triggered browning on black

garlic. The Maillard reaction which involves a reaction between reducing sugar and free amine group is the main key to the production of black garlic, where this reaction will accelerate along with increasing temperature and decreasing water content (Sailah and Miladulhaq, 2021). This reaction occurs along with polysaccharide enzymatic and non-enzymatic hydrolysis during aging (Shang et al., 2019). Thermal process also affects alliinase activation to catalyze alliin into allicin, a precursor substance of organoleptic characteristics in black garlic (Chhabria and Desai, 2018).

All of organosulfur compounds reported changed during thermal processing. Lai et al. (2013) claimed 3 related bio-sulfur compounds to determine black garlic quality, these are S-allyl-L-cysteine (SAC), Alliin, and γ -glutamyl-S-allyl-cysteine (GSAC). When fresh garlic got chopped, Alliin roles as allicin precursor by interact with alliinase enzyme. The aging process affects alliinase activation to catalyze alliin compound transformation into allicin which will form typical organoleptic characteristics in black garlic (Chhabria and Desai, 2018). High temperature significantly affects allicin stability. As the temperature increase, It will degrade, decreasing allicin content and inhibit formation of pungent odor (Zhang et al., 2016).

Besides the usage of controlled temperature, a number of studies also revealed several important factors that impact to organosulfur changes during aging such as humidity, pretreatment, and aging period (Bae

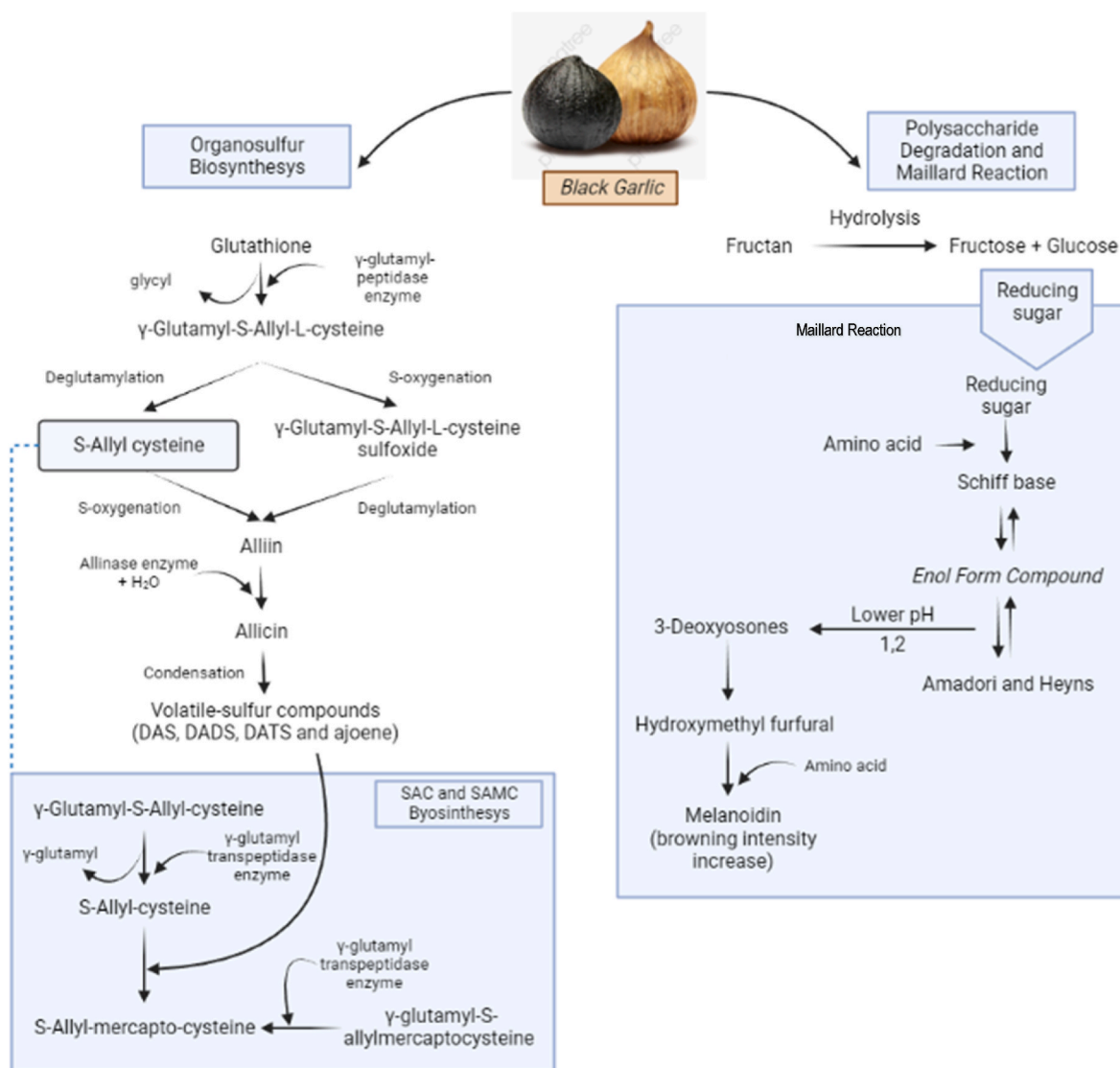


Fig. 1. Organosulfur biosynthesis, polysaccharide degradation and maillard reactions during aging in black garlic.

et al., 2014; Chen et al., 2020; González-Ramírez et al., 2022; Li et al., 2020; Zhang et al., 2015). Over these past years, black garlic researchers have shown chemical characteristics, quality, as well as the therapeutic effect results are influenced by the processing method. Most of the studies focused on the production optimization and its relationship within the biological activity using organosulfur compounds, polysaccharides, polyphenols, and other derivative products to determining black garlic quality. Although variety researches had done, black garlic production is not clearly completed to be standardized. Considering organosulfur effect on black garlic's quality and functionality properties, further studies about chemical reactions on organosulfur dan polysaccharides changes influenced by aging conditions are necessary to be explained. Therefore, this review will thoroughly discuss the changes in organosulfur characteristics during aging and its effect on the functional properties of black garlic.

2. Nutritional fact of garlic and black garlic

Black garlic has been processed through aging methods without any additive addition at high temperature (60–90 °C) and humidity (60–90%) for 10–90 days (Kimura et al., 2017). Black garlic often mentioned by the name of aged garlic. Black garlic has some difference with aged garlic extract (AGE), where AGE is made by soaking garlic slices in ethanol solution (15–20%) and extracted after being left to stand at room temperature for 10–18 months (Jing, 2020). Black garlic is the result of the aging process of garlic (*Allium sativum* L.) which is widely used and developed in Asian countries. Physicochemical changes of black garlic are strongly influenced by their conditions during thermal process (Aging), especially on polyphenols, flavonoids, and secondary metabolites such as organosulfur and antioxidant Maillard products (Choi et al., 2014).

Table 1 shown nutrition compounds in garlic have a difference levels form black garlic. Among the various chemical ingredients, organosulfur and polysaccharides are included and widely involved in the formation of black garlic. Organosulfur compounds use to be responsible as a precursor to the pungent taste of garlic as well as affecting the biological activity of black garlic (Yudhistira et al., 2022). In other hand, some case reports polysaccharides in garlic have a side effects to the human's digestion. During aging, it has a role in the formation of appearance characteristics, play an active role in the Maillard reaction, which is none other the dominant reaction in black garlic aging and biological

Table 1
Nutrition comparison between fresh garlic and black garlic.

Nutrition Compound	Fresh Garlic	Black Garlic	Reference
Moisture content	0.98%	0.93%	Toledano Medina et al.. (2016)
Water-soluble sugar	5.9 g/kg	472.4 g/kg	Martínez-Casas et al.. (2017)
Lipid	0.2%	0.60%	Ahmed & Wang. (2021)
Protein	0.9%	1.2%	Lu et al.. (2017)
Amino acid	19.43 ± 0.01 mg/g	14.86 ± 0.01 mg/g	Lu et al.. (2017)
Organic acid	16.70 ± 0.61 g/kg	64.50 ± 7.55 g/kg	Zhang et al.. (2016)
Vitamin	6.92 ± 0.02 g/kg	9.26 ± 0.03 g/kg	Ahmed & Wang. (2021)
Mineral	11.74 ± 0.02 g/kg	13.14 ± 0.03 g/kg	(Kang, 2016b)
Ash content	73.59 ± 0.89 mg/100g	75.36 ± 0.02–114.36 ± 8.65 mg/100g	(Kang, 2016b)
Pyruvat	49.05 ± 1.2 mmol/100g	264.02 ± 2.4 mmol/100g	Jeong et al.. (2016)
Volatil compound	49.76 µg/g	39.04–100.46 µg/g	Setiyoningrum et al.. (2021)
Carbohidrat	22.91 ± 0.03–23.7 ± 0.3 g/100g	46.6 ± 0.55–51.28 ± 0.07 g/100g	Tahir et al.. (2022)

activity (Persaud, 2022; Ríos-Ríos et al., 2018). Therefore, both substances along with other compounds are the important parameters in determining black garlic's quality.

3. Organosulfur and polysaccharide characteristic of black garlic

The health benefits in garlic are generally associated with variety organosulfur compound and have been identified as the mayor biological substances in black garlic as shown in Table 2 (Kim et al., 2018; Liu et al., 2022). Garlic basically contain more than 20 types organosulfur compounds which are classified into 2 types, water-soluble sulfur compounds consisting of Alliin, S-ALLYLCYSTEINE (SAC), and S-allyl Mercapto Cysteine (SAMC) and oil-soluble sulfur compounds consisting of diallyl thiosulfinate (Allicin), diallyl sulfide (DAS), diallyl disulfide (DADS), diallyl sulfide (DADS) and trisulfide (DATS), and e/z-ajoene (Shang et al., 2019). However, organosulfur content changed due to reactions that occur during spontaneous fermentation, causing the increase and decrease of each type of organosulfur black garlic.

The fermentation process in black garlic at a temperature of 80 for 15 days can significantly increase antimicrobial activity. The results of this study show a significant increase in the antibacterial properties of black garlic against *Streptococcus mutans* and *Enterococcus faecalis* (Halimah et al., 2021). Antimicrobial activity has been proven in black garlic. This is because there are compounds derived from S-allyl cysteine (SAC), namely allyl sulfide, dallyl disulfide (DADS), and dallyl trisulfide (DATS). Has been proven to work as a bactericide. This compound can fight antibiotic-resistant bacteria such as *Shigella dysenteriae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Eshceria coli*, *Streptococcus* spp, *Salmonella* spp, and *Proteus mirabilis*.

The antimicrobial activity of black garlic is even higher than fresh garlic, this is because there is a change in the allicin compound into the S-allylcystein compound which is a component that forms allin, then the allin will be converted into the allinase enzyme to become allicin which has antibacterial activity. Therefore, the antimicrobial activity of black garlic is greater than that of fresh garlic (Kimura et al., 2017).

Black garlic shows antibacterial activity, namely bacteriostatic capacity against the bacteria *Bacillus subtilis*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* as well as *Escherichia coli*. Research (Chang and Jang, 2021) shows that the diameter of the resulting inhibition zone has a larger diameter in black garlic with a bacteriostatic capacity against gram-positive bacteria that is greater than gram-negative bacteria. Black garlic has high antibacterial activity, this is due to the presence of the compound allicin, but there are also other compounds that act as antibacterials, namely the content of antimicrobial peptides which are known to be found in black garlic.

Table 2
Organosulfur comparison between fresh garlic and black garlic.

Compound	Fresh Garlic	Black Garlic	Reference
Allicin	3.18 ± 0.11 g/kg	Not detected	Zhang et al.. (2015)
Alliin	11.28 ± 0.22 g/kg	2.31 ± 0.07 g/kg	
S-allyl cysteine	19.61 ± 0.35 µg/g	105.07 ± 27.73 µg/g	Bae et al.. (2014)
Allyl sulfide	Tidak terdeteksi	53.93 µg/g	Setiyoningrum et al.. (2021)
Diallyl disulphide	591.67 µg/g	62.84 µg/g	
Diallyl trisulfide	Not detected	961.76 µg/g	

3.1. Water-soluble sulfur compound

3.1.1. Alliin

Alliin is the largest sulfur-amino acid compound (about 1.29% of the total nutritional components) in garlic. Alliin belongs to the intermediate compounds of allicin as well as precursors for other sulfur compounds. When tissue damage occurs in garlic, alliinase will be released and rapidly hydrolyses alliin to become allicin (Chen et al., 2017). Alliin has a weak sulfoxide bond so that it is susceptible to degradation during the aging process of black garlic (Yoshimoto and Saito, 2017). Alliin can be applied medically as a bioactive compound with antioxidant properties, antimicrobial, has a hypolipidemic activity, anti-inflammatory, and uses as anti-cardiovascular effect (Chen et al., 2017; Zhang et al., 2015). Furthermore, Alliin compound also used as the quality indicator of black garlic.

Alliin is the main thiosulfinate found in fresh garlic which has a distinctive taste and aroma. allicin is unstable at near neutral PH, and can be easily degraded at high PH (García-Villalón et al., 2016). Alliin becomes an unstable compound in fresh garlic but is converted into a stable compound, namely SAC, which functions as an antioxidant in black garlic. Fresh garlic contains 2% alliinase protein as an enzyme. After going through the process of making black garlic, alliinase lyses cytotoxic cysteine sulfoxide or alliin to form cytotoxic alkyl and thio-sulfate alkanes such as allicin. The allicin in black garlic contributes to the characteristic taste and characteristics of garlic. After that, allicin will break down into another compound, namely SAC, through a catabolism pathway other than the alliin-allicin pathway (Colín-González et al., 2015).

3.1.2. S-allyl cysteine (SAC)

S-Allyl cysteine (SAC) is an amino acids-water soluble organosulfur predominantly contained in black garlic and has a variety of biological activities such as antioxidant, anti-inflammatory, anti-obesity, cardioprotective, and hepatoprotective activity (García-Villalón et al., 2016; Malaphong et al., 2022). SAC available in white crystalline powder, non-hygroscopic, stable, and safer to be consumed at high concentrations than other garlic organosulfur compounds unlike allicin and diallyl sulfide group (Colín-González et al., 2012; Xu et al., 2015). Moreover, SAC is generally produced by hydrolyses reaction of γ -glutamyl-S-allyl-L-cysteine (GSAC) by the activation of enzyme γ -glutamyl transferase (γ -GTP, EC 2.3.2.2). However, SAC is also thought to result from the reduction of alliin due to an increase in temperature that cannot be tolerated by γ -GTP (Yudhistira et al., 2022).

Garlic contains as much as 20–30 μ g/g SAC and increase more than 6 folds through the thermal process at a certain time (black garlic aging). The SAC has been analyzed by some researchers previously used HPLC-UV (ultraviolet detection) method. Nevertheless, it is known that the compound of maillard products can absorb UV rays then diffuse with SAC, causing difficulty in obtaining accurate SAC analysis results. Therefore, the analysis method chosen for SAC uses alternative HPLC-FLD (Fluorescence detection) with more stable, sharp, and symmetrical analysis results (Bae et al., 2014).

3.1.3. S-allyl mercapto cysteine (SAMC)

SAMC is a stable, tasteless, odorless organosulfur extracted from aged garlic or black garlic extract. SAMC levels in black garlic could increase up to 6 times compared to fresh garlic. SAMC is produced by the transformation of allyl sulfide compounds from allicin which has low stability to high temperatures. Studies have shown that SAMC has a hepatoprotective effects on acute liver disease, non-alcoholic fatty liver disease (NAFLD) and liver cancer and it is able to fight cancer cells in the stomach, intestine, prostate, ovarian, and thyroid cancer (Luo et al., 2021; Ríos-Ríos et al., 2019).

3.2. Oil-soluble sulfur compound

3.2.1. Diallyl thiosulfinate (allicin)

Allicin is one of the most organosulfur compounds produced from the conversion of non-protein amino acids, alliin as a substrate with the alliinase when garlic tissue is physically damaged (Reiter et al., 2020). This compound represents 70–80% of the sulfur component and is responsible for the very strong taste and aroma of garlic (Salehi et al., 2019). Allicin belongs to the reactive sulfur components that bound to thiol groups such as glutathione or Cysteine proteins (Gruhlke et al., 2019). In addition, allicin is highly reactive to temperature, oxygen, organic solvents, and pH changes. These factors force this compound susceptible transformation into another organosulfur derivative forms (mostly diallyl sulfide, diallyl disulfide, diallyl trisulfide, ajoenes, and vinylidithiins) (Tavares et al., 2021). Allicin has been categorized as a high antimicrobial compound in garlic by Cavallito and Bailey in 1944. In addition, allicin has been shown inhibitory effects at resistance microorganisms (bacteria, molds, and oomycetes) to various types of chemical drugs, induce apoptosis, and proliferation cell at sub-lethal dose (Gruhlke et al., 2019).

3.2.2. Diallyl sulfide (DAS)

Diallyl sulfide (DAS) is a lipophilic thioether form by the oxidation of alliin which is a direct precursor of the distinctive taste and aroma of garlic. DAS along with allyl methyl sulfide (AMS) are known to be effective as antioxidants in arterial structural changes caused by hypertension (Mikaili et al., 2013). The health benefit of DAS is also believed to have pharmacological effects in preventing cancer of the mammary gland, stomach, intestines, and lungs. In a low concentration, DAS identified as non-toxic compound but could be potentially dangerous when consumed at ≥ 1600 mg/kg dose. Dutta et al. (2021) reported toxicity effect of DAS was triggered by its role which rapidly oxidized into intermediates, diallyl sulfoxide (DASO) and diallyl sulfone (DASO₂) that suspected as toxic compounds. Therefore, they define garlic's lethal dose of 50 (LD50) for garlic extract on mice around 0.5 ml/kg to 30 ml/kg. Although DAS content still exist in black garlic, the capacity itself decreased along with the level of allicin as much as 1.4% (Molina-Calle et al., 2017).

3.2.3. Diallyl disulfide (DADS)

Diallyl disulfide is one allyl compounds contained in garlic oil and widely used as a flavoring agent and traditional medicine. This compound is produced from allicin compounds that are converted because they are unstable to temperature changes, along with other volatile allyl sulfides (Colín-González et al., 2012). DADS are known to have antioxidant and antitumor activity. DADS is also able to inhibit thrombosed aggregation, improve macrophage function, and increase hypolipidemic activity so it is applied as a drug for microbial infections, hypolipidemia, and a drug for heart disease (Shin et al., 2013).

3.2.4. Diallyl trisulfide (DATS)

Diallyl trisulfide (DATS) is another active component of garlic that has antithrombotic, anticoagulant, and antiplatelet effects. A study reported DATS and DADS can encourage immunomodulatory activity through increased calcium influx and are anti-inflammatory in mouse samples by increasing lymphocyte and monocyte levels (Malla et al., 2022). Not only as immunomodulatory, DATS also able to suppress the growth of cancer cells in culture and in vivo by trapping the cell cycle and induction of apoptosis (Subramanian et al., 2020).

4. Polysaccharide and other related compounds

Garlic as black garlic's source contains as much as 75% carbohydrates (per dry weight) with fructans as the largest polysaccharide compound among other saccharides (Table 3). Fructans or fructooligosaccharides (FOS) are indigestible carbohydrates that helps to promote

Table 3

Polysaccharide and other related compounds comparison between fresh garlic and black garlic.

Compound	Fresh Garlic	Black Garlic	Reference
Fructan	550–600 mg/g	>100 mg/g	Yuan et al. (2018)
Fructose	9.36 ± 0.13 g/100g	31.05 ± 1.34 g/100g	Najman et al. (2021)
Glucose	2.12 ± 0.05 g/100g	4.84 ± 0.28 g/100g	
Sucrose	0.02 ± 0.00 g/100g	3.11 ± 0.12 g/100g	
5-Hydroxymethyl furfural (HMF)	Unidentified	0.23 ± 0.04 g/kg	Li et al. (2015)
Melanoidin	<0.1 OD	±1.8 OD	Yuan et al. (2018)
Amadori	Unidentified	280,56–762,53	Yuan et al. (2016)
Heyns		µg/g	

Desc: OD (Optical Density).

humans health potential. Fructans in garlic belong to the ketose group and are composed of fructose and glucose (14:1 ratio) (Yuan et al., 2018). The main chain fructan is composed of (2 → 1)-β-D-fructopyranose bound with (2 → 1)-α-D-glucopyranose at the ends of the non-reducing chain branches (2 → 6)-β-D-fructopyranose (Chen et al., 2013). Fructans in plants contain various types based on their chemical structure and polymerization degrees (DP). The distribution of fructans depends on environmental conditions and plant growth (Verma et al., 2021).

Fructans is one of the important factors to the quality of black garlic (Lu et al., 2018). It will be hydrolyzed by endogenous fructan exohydrolase (FEH) enzyme into sucrose and fructose (Verma et al., 2021). FEH optimally active at 45 °C degree and pH 5.5. Fructans and FEH then interact and produce several decomposition products including oligo-fructose, fructose, and glucose (Cheong et al., 2012). However, Lu et al. (2018) declared that polysaccharide levels were lower during aging is strongly caused by thermal treatment, where reducing sugar content were higher at 90 °C than 70 °C. It can be concluded that although FEH undergoes inactivation at 70 °C temperature, total reducing sugar still continue to increase during thermal process (F. Li et al., 2020).

Most studies proved polysaccharides degraded into several saccharides form during aging, consisting of glucose, fructose or disaccharides (N. Li et al., 2015). This statement is supported by Li et al. (2017) who reported a decrease of approximately 30% total polysaccharide during black garlic production into monosaccharide constituents namely fructose, galactose, and galacturonic acid with a molar ratio of 307:25:32. Liang et al. (2015) also showed a significant depreciation of fructose content within 5th to 25th day based on the increase of fructose levels. Analysts concluded that lower fructose level is caused by high temperature and low pH aging conditions that promote sucrose to hydrolyzed and produce glucose and fructose. The result of this hydrolysis reaction also formed the texture and sweet taste on black garlic. Black garlic also has a sense of acidity caused by increased organic acids resulting from polysaccharide degradation at the same time (Ahmed and Wang, 2021).

Black garlic contains reducing sugar 30–80 times higher than fresh garlic (Z. Qiu et al., 2020). Martínez-Casas et al. (2017) confirmed each monosaccharide in the purple black garlic sample increased, such as fructose (0.38 ± 0.06 g/100 g DM to 44.73 ± 4.41 g/100 g) and glucose (0.21 ± 0.02 g/100 g DM to 2.51 ± 0.24 g/100 g). The monosaccharides formed after the degradation process are subsequently used as substrates (reducing sugars) in Maillard reaction, where the reducing sugars will interact with amino group of the lysine residue on peptide or protein (Kang, 2016a).

The Maillard reaction that occurs during aging triggered protein denaturation and forms a number of amino acids along with the production of several secondary metabolite compounds such as amadori, heyns, melanoidin, and 5-HMF. According to USDA (2018), garlic

contains 1.5–2.1% protein. When Maillard reaction runs, at the same time degradation of proteins or peptides do exist from enzymatic and non-enzymatic hydrolysis in acidic conditions, then resulting an increase in several types of amino acids in black garlic such as L-alanine, L-valine, L-isoleucine, L-tyrosine and L-phenylalanine (Liang et al., 2015). However, Li et al. (2015) showed the opposite result, where amino acids in black garlic samples treated with freezing pretreatment decreased by 50.97%. This makes the levels of amino acids in black garlic can vary depending on the type and treatment of aging given.

Amadori and heyns are the first 2 major compounds of the Maillard reaction resulting from the re-formation of amino acids and reducing sugars. Both compounds are important and are commonly used as indicators of the quality of the maillard reaction (taste, color, and functionality) as well as acting as melanoidin precursors. Amadori and heyns in black garlic also contribute to the formation of furosine (2-furoylmethyl-lysine, 2-FM-Lys) and 2-furoylmethyl amino acids (2-FM-AA) through acid hydrolysis so that both compounds are also important indicators in assessing the quality of black garlic (Andruszkiewicz et al., 2020; Ríos-Ríos et al., 2018).

Melanoidin compounds are the end products of the maillard reaction which belong to carbohydrates with polymerized nitrogen content, Brown in color, and have a high molecular weight. Melanoidin is also often referred to as a brown pigment at the same time that makes Black garlincs have a brownish color when experiencing the aging process (Cui et al., 2021; Z. Qiu et al., 2020). In addition to melanoidins, non-enzymatic browning also produces 5-Hydroxymethyl furfural (5-HMF), an aromatic compound with 5 carbon rings as one of the important antioxidant components in black garlincs and is strongly influenced by storage conditions including temperature and pH (Kimura et al., 2017). These compounds also enhance the antioxidant, anti-inflammatory, and antitumor activity of black garlic (Li et al., 2015).

5-HMF is produced from fructose and glucose during thermal processing and is used as an indicator of the presence of carbohydrates in a food. 5-HMF which is an acyclic aldehyde formed from hexoses through acidic conditions. This compound is one of the Maillard reaction products and caramelization formed from 3-deoxyglucosone (3-DG), which is a compound resulting from the dehydration of 1,2-enolization of glucose and fructose and through the separation of the amine group on the Schiff base during the formation of the Maillard reaction (Hu et al., 2022). The formation of 5-HMF is affected by temperature and pretreatment. Through NMR analysis, 5-HMF content is known to increase up to 6-fold at a temperature of 70–28 days and 25 days (Liang et al., 2015; Nakagawa et al., 2020).

HMF could also formed through caramelization reaction without amine groups. Zhang et al. (2016) added that higher the content of 5-HMF will rise the browning intensity of black garlic with a maximum content in 5 g/kg. This indicates that 5-HMF also contributes to influencing the color of black garlic. Both polysaccharides and Maillard products have a large role in black garlic's characteristics and nutritional content. Caramelization has its own role but both Maillard reaction and polysaccharide degradation are still be the dominant reactions and play an important role in black garlic production.

The fermentation method in black garlic involves the role and activity of the pure isolate found in black garlic during the fermentation process and a certain temperature and time duration that occurs with spontaneous fermentation. During fermentation, sulfur compounds are produced which are produced by the microbes involved, resulting in a light and distinctive aroma without being as pungent as fresh garlic. So black garlic is considered to be able to eliminate it (Z. Qiu et al., 2020) a pungent odor that is undesirable and detrimental to digestion and irritation. Apart from that, optimal fermentation must depend on the appropriate temperature and time. at a fermentation temperature of 85–70 with a fermentation time duration of 5–6 days and 11–13 days produces black garlic with uniform black-brown color characteristics, good taste, light and not pungent odor, 30% reduction in soluble sugar

content, as well as a 15 times increase in total phenolic content and SOD activity (Lu et al., 2017).

Aging method in the process of making black garlic with high temperature and high humidity. However, several studies have used varying aging conditions to obtain optimal conditions to maximize the properties and activity of antioxidants. In the aging process, many important components increase, for example polyphenols and flavonoids, as well as Maillard reaction intermediates which are known as antioxidant agents. In the aging method using a temperature of 60, the color of black garlic is not uniform and not perfect, but after a temperature of 70 the color is uniform black-brown, at 90% humidity with a fermentation time of 35 days, it shows higher antioxidant properties (Abdullah et al., 2019).

5. Changes in organosulfur during aging

Aging treatment or some other may call it spontaneous fermentation in black garlic processing initiate some reactions both enzymatic and non-enzymatic (Lu et al., 2017). The thermal process then has a major impact on changes in the characteristics and chemical compounds in black garlic, one of which is organosulfur compounds and produces different functional properties compared to fresh garlic (Najman et al., 2022; Tran et al., 2019). One of the reactions that plays a role during aging is the Maillard reaction. The Maillard reaction occurs when there is an interaction between the free amino acid group, peptide or protein, and carbonyl group of reducing sugar during thermal processing (Gamboa-Santos et al., 2014). Reducing sugars availability as Maillard's substrates is obtained from the degradation of polysaccharides at high temperatures (F. Li et al., 2020).

Maillard reaction affects chemical and functionality changes during aging. Nevertheless, this reaction is very susceptible to several factors, including time, temperature, humidity, reactant source, concentration and type of reducing sugar, amino acids, pH, and food content itself (Ogotu et al., 2017). Based on the evidence (Cui et al., 2021; Rini, 2016; V. Verma et al., 2020), Maillard reaction is generally divided into 3 phases, such as:

1. Initial Step

It is the first stage of Maillard reaction, where a reaction occurs that caused by the addition of a carbonyl group from the open chain of the reducing sugar to the main amino acid group. Amino acid groups then dehydrated to produce Schiff base compounds. Schiff's base will undergo a further process which ultimately forms 2 intermediate compounds as organoleptic precursors, namely amadori (aldose-shaped sugars) and heyns compounds (ketose-shaped sugars).

2. Intermediate Step

It is the decomposition stage of Arp (Amadori Rearrangement Product). This stage involves several reactions such as rearrangement of low molecular weight compounds, fragmentation, cyclization, and stecker degradation (interaction between amino acids and carbonyl compounds). The end result in this intermediate stage in the form of aldehyde, ketone, dicarbonyl, and heterocyclic components.

3. Final Step

Products from the intermediate stage then further converted in the final stage of Maillard reaction. Compounds such as carbonyl and dehydroreductons will be converted into melanoidin pigmen. Melanoidin has a role as flavor and color precursor in black garlic. It can be interpreted that melanoidin levels is directly proportional to the rate of browning intensity. Browning degree is strongly influenced by high temperature and aging time. Longer time and higher temperature used in aging, melanoidin levels will increase and darken the color of black

garlic (Toledano Medina et al., 2016).

Maillard reaction also produces other chemical substances. Amadori and Heyns are intermediates compound that are produced in the Maillard reaction and involved in aging process. Amadori acts as a precursor of flavor-agent compound while Heyns plays a role in increasing black garlic's antioxidant activity. Heyns compounds are actually derived from a reaction between fructose and amino acid (Yuan et al., 2016). Compared to glucose, fructose when interacted with amino acid on black garlic has create greater antioxidant levels, so both compounds play an active role during aging. The catalytic dehydration between reducing sugars and amino acids in also forms another intermediate compound, 5-Hydroxymethyl furfural (5-HMF).

Li et al. (2020) divided polysaccharide degradation into 3 stages. The first stage begins with fresh garlic pretreatment, where in this phase the cell structure is damaged and promoted contact between enzymes and substrates. On the 0th to 3rd day of aging, the second stage then occurs. This stage of polysaccharide degradation is still dominated by enzymatic reactions by Endogenous Fructan Exohydrolase (FEH) enzyme at 45 °C. Furthermore, entering the third stage, degradation continues to increase after the 3rd day due to increased aging temperature. This is also what makes reducing sugar production continue to increase.

Polysaccharides contained in garlic will degrade with increasing temperature and aging time. A large number of researchers have linked the activity of endophytic microorganisms to carbohydrate degradation. Endophytic microorganisms live in the intercellular of plants and have a symbiosis of mutualism with their hosts. Plants will provide nutrients to be processed by microorganisms into bioactive components that brings benefits to the plant (Abdulmyanova et al., 2016). Microorganism community, usage of temperature and fermentation time affect the number and diversity of garlic and black garlic endophytes. Most of the endophytic bacteria in black garlic are identified as *Bacillus subtilis*, followed by *Bacillus licheniformis*, *Bacillus methylotrophicus* and *Bacillus amyloliquefaciens* (Lestari et al., 2021; Z. Qiu et al., 2018). Research on endophytic fungi shows different result. Although Shentu et al. (2014) had successfully isolated *Trichoderma brevicompactum* as the dominant strain in garlic based on morphological characteristics and nucleotide sequences, there are no further studies that identify the activity of endophytic mold in black garlic.

Although enzymatic and non-enzymatic browning are a crucial mechanism due to the black garlic's formation, *Thermus* and *Bacillus* play a role in increasing reducing sugar levels, total phenol, and total acidic in black garlic (Kang, 2016b; Z. Qiu et al., 2018). The large number of total acidities during aging process is caused by an increase in reducing sugar content, glucose, which occurs through fermentation process to produce acetaldehyde by the alcohol dehydrogenase enzyme. Acetaldehyde then oxidized to acetyl-coenzyme A (coA) by the enzyme aldehyde dehydrogenase and dephosphorylated to acetic acid by the enzyme acetate kinase. In addition, this fermentation process also produces various other types of organic acids such as lactic acid, lactic acid, formic acid, propionic acid, glycolic acid, fumaric acid, and succinic acid (Lestari et al., 2021). Microorganisms are suspected to influenced the black garlic's flavor.

Black garlic production is not only involves browning reactions but also some chemical changes by enzymatic reactions, such as γ -glutamyl cysteine catabolism which also supports the increase in biological and antioxidant activity of black garlic (Colín-González et al., 2012). Fresh garlic contains variety organosulfur compounds that responsible in producing garlic's unpleasant flavor and odor characteristic. It is caused by the presence of allicin as the dominant thiosulfinate compound in garlic produced from alliin compounds. When garlic got physically damaged, alliinase in the vacuole tissue will be activate and quickly hydrolyze alliin into allicin. Allicin then be condensed into sulfur-producing compounds characteristic of garlic due to its low thermal stability bond (Janska et al., 2021; Yoshimoto and Saito, 2017). Allicin decomposed into dipropyl disulfide, diallyl tetrasulfide, DADS, diallyl sulfide, diallyl trisulfide, ajoenes, and vinylidithiins (Yudhistira

et al., 2022).

Alliin content in garlic will be decreased along with the increase in temperature during aging. Alliinase enzyme has a resistance to heat temperatures above 60 °C. When the aging temperature reaches more than 60 °C, alliinase will be inactivated and cause degradation of alliin and alliin (Ahmed and Wang, 2021; Zhang et al., 2015) has found during the black garlic processing at 70–80 °C for 10 days, alliin content decreased by as much as 80%. This could be caused alliin sulfoxide bond being unstable due to the high temperatures.

Alliin in garlic during the heating process at a temperature >60 °C identified changes into secondary organosulfur components, namely S-allyl-L-cysteine (SAC) and some ether compounds include allyl alanine disulfide, allyl alanine trisulfide, allyl alanine tetrasulfide, dialanine disulfide (cysteine), dialanine trisulfide and dialanine tetrasulfide (Chen et al., 2017). These compounds will eventually transform back into S-allyl-mercapto-cysteine (SAMC) which is stable, tasteless and odorless and consist 6 folds higher in black garlic. Most studies connect the occurrence of SAMC high-levels during heating with the alliin conversion (Ríos-Ríos et al., 2019).

In the other hand, SAC as one of the major sulfur compounds in black garlic is generated from the enzymatic hydrolysis reaction of γ -glutamyl-S-allyl cysteine by γ -glutamyl transpeptidase enzyme (γ -GTP). γ -GTP has an optimum activity until 40° and recognize to completely inactive at 75 °C after 30 min aging (Xu et al., 2015). In addition, Bae et al. (2014) successfully identified the growth of SAC at a 85 °C thermal processing supported by high water content through alliin reduction. The production of bioactive compounds in black garlic such as SAC is generally carried out by enzymatic reactions and could continue forming in aging condition though enzymatic activity is not possible at high temperatures (above the enzyme's maximum thermal degree tolerance).

6. Aging conditions factors affecting black Garlic's quality

Black garlic's production can be influence directly by numerous aging conditions as shown in Table 4. In this review, we divided several factors into 4 parameters, including temperature and humidity, aging period, microorganism activity, and pretreatment application.

6.1. Temperature and humidity

Temperature and humidity are 2 important factors as determinants of the final quality of black garlic (Jing, 2020). Based on Table 5 above, it can be seen that the difference in temperature and humidity is related to the formation of the psychochemical content of black garlic. Some of the research results above show that the water content and pH of black garlic will decrease with increasing aging temperature (Bae et al., 2014; Choi et al., 2014; Dewi, 2018; González-Ramírez et al., 2022; Kang, 2016a, 2016b; Najman et al., 2021; Toledano Medina et al., 2016; Zhang et al., 2016). This water content and low pH make black garlic have a longer shelf life than fresh garlic (Bae et al., 2014).

The water content of black garlic is inversely proportional to the temperature. Considering the final texture, (Zhang et al., 2016) states that the water content value to produce the best soft texture is in the range of 40–50%. Black garlic will look tough when the water content is >35%. In addition, humidity also has an effect on reducing water content (Toledano Medina et al., 2016). During aging, the pH of garlic consistently drops from ± 6 to a pH range of 3–4 (Bae et al., 2014; Choi et al., 2014; Kang, 2016b; Toledano Medina et al., 2016). The decrease in pH has a correlation with the formation of carboxylic acids in the Maillard reaction which also triggers an increase in the total acid content (5 g/kg 31–38 g/kg) which causes the sour taste of black garlic (Ríos-Ríos et al., 2018).

Fresh fructan as the dominant polysaccharide abundant in garlic decreased by 84.79% due to degradation when entering the aging phase due to its unstable nature to the increase in environmental temperature accompanied by an increase in glucose and fructose by 3–4 times

(Najman et al., 2021). Maillard reaction is very influential on temperature and humidity (Ogutu et al., 2017). The degradation of polysaccharides (fructans) at low temperatures (>70 °C) tends to be caused by enzymatic hydrolysis by the FEH enzyme. Although this enzyme is inactivated at temperatures above 70 °C, the decrease in polysaccharide content in black garlic is largely influenced by the thermal process, so it appears at a temperature of 85–90 °C (Lu et al., 2018; Z. Qiu et al., 2020). Another opinion shows that at a temperature of 55 °C it can produce high levels of reducing sugar if the humidity used is high (80%) (González-Ramírez et al., 2022; Yuan et al., 2018).

At the same temperature (55 °C), the higher the aging RH, the higher the fructose and glucose levels obtained. This increase can be caused by increased molecular mobility and triggers increased reactivity as an important factor for the success of a non-enzymatic browning. The reducing sugar content is in line with the browning intensity. The maximum rate of production of maillard compounds in black garlic is at medium humidity, which is around 71–90% (depending on temperature and aging time) (Bell, 2020; González-Ramírez et al., 2022; Taoukis and Richardson, 2020). The higher the temperature and humidity, the darker the intensity of the brown color of the black garlic which comes from the large production of melanoidin compounds (Kang, 2016a).

5-HMF production is slow at 60 °C. The greater the production of reducing sugar produced, the greater the derivative products of the Maillard reaction (amadori, hens, melanoidin, 5-HMF compounds) and amino acids produced (Choi et al., 2014; Liang et al., 2015; Ríos-Ríos et al., 2019). Overall, quality black garlic is produced at an aging temperature of 70–80 °C. Aging at 90 °C (>80 °C) is able to run faster but has a bitter and sour taste. On the other hand, black garlic is not fully ripe at 60 °C unless it is balanced with high humidity (Kimura et al., 2017; Toledano Medina et al., 2016).

The pungent taste and aroma of garlic disappears with the thermal process due to a decrease in alliin, alliin compounds and the production of SAC compounds and other organosulfur compounds (Bae et al., 2014; Liang et al., 2015; Zhang et al., 2016). Zhang et al. (2015) in his study found an 8-fold decrease in alliin compounds caused by the Maillard reaction and the conversion of alliin into other organosulfur products (such as SAC and SAMC) in the temperature range of 70–80 °C. The high content of SAC at an aging temperature of 40 °C compared to 85 °C is associated with the hydrolysis activity of the γ -GTP enzyme which is still active (Bae et al., 2014). This enzyme will become inactive when the temperature is 70 °C but the SAC level can still be increased through structural modification. The increase in the content of SAC and other derived organosulfur compounds can be supported by the availability of moisture content from humidity (Park et al., 2014).

The use of a temperature of 70 °C is also considered better because the alliinase enzyme is completely inactivated so that alliin is no longer produced Liu et al. (2022) which showed that the enzymatic activity and composition of SAC, Alliin, and γ -glutamyl cysteine as part of the quality parameters of black garlic showed the best values at 70 °C and 80% RH. The content of SAC and other organosulfur related to the antioxidant activity and functionality of black garlic. The antioxidant levels of black garlic increased due to the increase in several antioxidant compounds such as organosulfur compounds (SAC, SAMC, DAS, DADS, and DATS), 5-HMF, amadori, heyns, melanoidin, ascorbic acid and polyphenols. Antioxidant capacity and other functional compounds (Azhar et al., 2021; Bae et al., 2014; Choi et al., 2014; Dewi, 2018; González-Ramírez et al., 2022; Kim et al., 2013; Liu et al., 2022; Thalia et al., 2020; Toledano Medina et al., 2016; Yu et al., 2020).

Some result shown black garlic has a higher antioxidant activity compared to fresh garlic at 70 °C and 90% humidity for 35 days (Choi et al., 2014). In addition, Sun and Wang (2018) also concluded that samples of black garlic with the best taste, psychochemical and antioxidant content were overall obtained in samples with a temperature of 70 °C and 85% humidity for 8 days of aging.

Table 4
Variations in temperature, humidity, and aging time and changes in characteristics of black garlic.

No	Type	Aging Conditions	Physical and Nutritional Composition	Polysaccharides and Maillard Products	Organosulfur	Antioxidant	Other compounds	Reference
1	Fresh Korean garlic	Temperature: (1) 40 °C (2) 55 °C (3) 70 °C (4) 85 °C RH: 70% Time: 45 Days	<ul style="list-style-type: none"> Decreased moisture content Lower pH at higher temperatures 	Increased browning intensity	SAC at 40 °C higher than 85 °C	<ul style="list-style-type: none"> DPPH increase Free radical scavenging is higher at 85 °C than at 40 °C Reducing power increases Increased electron donor ability 	–	Bae et al. (2014)
2	Fresh Korean garlic	Temperature: 70 °C RH: 90% Time: 35 days	<ul style="list-style-type: none"> Decreased pH Total acid increases Increased moisture content 	Increased reducing sugar content	–	<ul style="list-style-type: none"> DPPH increase ABTS increases until the 21st day and decreases on the 35th day FRAP increase 	–	Choi et al. (2014)
3	Korean garlic	Temperature: (1) 90 °C (2) 60 °C (3) 75 °C (4) 70 °C (5) 65 °C Rh: (1) 100% (2) 60% (3) 70% (4) 60% (5) 50% Time: (1) 34 h (2) 6 h (3) 48 h (4) 60 h (1) (5) 192 h	<ul style="list-style-type: none"> Decreased moisture content Increased ash content Decreased pH 	<ul style="list-style-type: none"> Melanoidin increases Increased browning intensity Total reducing sugars increase 	–	–	–	(Kang, 2016a, 2016b)
4	Garlic laiwu (chinese)	Temperature: (1) 60 °C (2) 70 °C (3) 80 °C (4) 90 °C RH: 80% Time: 72 days	<ul style="list-style-type: none"> The higher the temperature the faster the maturity occurs The best moisture content is at 70 °C The highest amino acid content is at 70 °C The highest total acid value is at 70 °C 	<ul style="list-style-type: none"> Browning intensity at 90 °C is higher than 60 °C The highest reducing sugar content is at 70 °C The highest HMF content is at 80 °C 	The lowest allicin content is at 60 °C and the highest at 80 °C	–	–	Zhang et al. (2016)
5	Fresh garlic	Temperature: (1) 72 °C (2) 75 °C (3) 78 °C RH: ±90% Time: (1) 78 °C: days 5, 10, and 14 (2) 75 °C: days 7, 14, and 21 (1) (3) 72 °C Day 11, 24, and 33	<ul style="list-style-type: none"> Decreased pH Decreased moisture content 	Increased browning intensity	–	Increased antioxidant capacity	Total polyphenols increase	Toledano Medina et al. (2016)
6	Fresh garlic	Temperature: 70 °C RH: -Time: (1) 30 days (P1) (2) 40 days (P2) (3) 60 days (P3) (1) (4) 90 days (P4)	<ul style="list-style-type: none"> The longer the aging time, the lower the moisture content The longer the aging time, the higher the ash content The highest protein content is on the 60th day The longer the aging time, the higher the 	–	–	<ul style="list-style-type: none"> The longer the aging time, the lower the GAEAC value The longer the aging time, the lower the IC value of 50% 	The longer the aging time, the total phenols increase	Dewi (2018)

(continued on next page)

Table 4 (continued)

No	Type	Aging Conditions	Physical and Nutritional Composition	Polysaccharides and Maillard Products	Organosulfur	Antioxidant	Other compounds	Reference
			carbohydrate content					
	Fresh garlic	Temperature: 70–90 °C RH: 80% Time: 8 days	–	<ul style="list-style-type: none"> • Decreased FEH enzyme activity • Increased reducing sugar content • Increased browning intensity 	–	–	–	Lu et al. (2018)
	Fresh garlic	Temperature: 55 °C RH: 80% Time: 90 days	–	<ul style="list-style-type: none"> • Decreased fructans • Decreased fructose 	–	–	–	Yuan et al. (2018)
	Japanese garlic	Temperature: 70 °C RH: -Time: (1) 7 days (2) 14 days (3) 21 days (1) (4) 28 days	–	<ul style="list-style-type: none"> • Increased browning intensity • Increased HMF content 	–	–	Increased polyphenol content	Nakagawa et al. (2020)
	Fresh garlic (multi-cloves garlic)	Temperature: (1) 90 °C (2) 60 °C (3) 75 °C (4) 70 °C (5) 65 °C Rh: (1) 100% (2) 60% (3) 70% (4) 60% (5) 50% Time: (1) 34 h (2) 6 h (3) 48 h (4) 60 h (1) (5) 192 h	–	Amadori compounds are elevated, dominated by fru-arg and fru-met	–	Increased antioxidant activity (FRAP, ORAC, ABTS)	–	Yu et al. (2020)
	Fresh garlic	Temperature: (1) 60 °C (2) 70 °C (3) 80 °C RH:-Time: (1) 30 days (2) 35 days (1) (3) 40 days	The longer time and higher aging temperature, the lower protein content	–	–	The longer time and higher temperature, the antioxidant activity increases more	The higher temperature, the higher total phenolic content	Thalia et al. (2020)
	Fresh garlic	Temperature: 70 °C RH: 70–80% Time: 30–40 days	–	–	–	The longer the aging time, the lower the IC50 value (antioxidant activity is increasing)	–	Azhar et al. (2021)
	Fresh garlic	Temperature: 55 °C Rh: (1) 10% (2) 30% (3) 58% (4) 71% (5) 96% Time: 40 days	<ul style="list-style-type: none"> • Decreased moisture content • The higher RH, higher the amino acids • The higher RH, higher the organic acid 	<ul style="list-style-type: none"> • The highest fructose content is at RH 58% • The highest β-glucose levels were at RH 58% • The highest α-glucose levels are at RH 71% • The highest sucrose content is at RH 30% • The highest 5-HMF content is at RH 71% 	The highest alliin levels are at RH 10% and the lowest at RH 71%	–	–	González-Ramírez et al. (2022)
		Temperature: 70–80 °C RH:-Time: 10 days	–	<ul style="list-style-type: none"> • The reducing sugar content increases then decreases until the 10th day • Decreased polysaccharide levels • Decreased disaccharide levels 	<ul style="list-style-type: none"> • Alliin levels rise in mid-aging and decrease until the 10th day • Alliin levels decrease 	–	–	Zhang et al. (2015)

(continued on next page)

Table 4 (continued)

No	Type	Aging Conditions	Physical and Nutritional Composition	Polysaccharides and Maillard Products	Organosulfur	Antioxidant	Other compounds	Reference
				<ul style="list-style-type: none"> Increased monosaccharide levels 				
	Fresh garlic	Temperature: (1) 65 °C (2) 75 °C (3) 85 °C Rh: (1) 70% (2) 75% (3) 80% (4) 85% Time: 16 days	<ul style="list-style-type: none"> The best sensory values are at a temperature treatment of 75 °C, RH 85% on day 8 Total acid increases Increased protein levels 	<ul style="list-style-type: none"> Increased reducing sugar Total sugar increases Increased 5-HMF content 	<ul style="list-style-type: none"> Thiosulfinate decreases Alliin decline 	<ul style="list-style-type: none"> Total reduction capacity increased Superoxide radical scavenging ability increase DPPH increase 	Increased polyphenol content	Sun and Wang (2018)
	Fresh garlic	Temperature: 60 °C RH:-Time: (1) 15 days (2) 30 days (3) 45 days (1) (4) Control: 0th day	<ul style="list-style-type: none"> It is dense, chewy, tasteless, the color is getting deeper black, and it has a sweet taste The longer the aging time, the higher the crude protein level The longer aging time, the less carbohydrate 	–	–	–	–	Nelwida et al. (2019)
	Fresh garlic	Temperature: 70 °C RH: 85% Time: 35 days	<ul style="list-style-type: none"> Decreased pH Decreased moisture content Decreased amino acids 	–	Alliin decrease	–	Total phenolic increases	Chang and Jang (2021)
	Fresh garlic	Temperature: 60–70 °C RH: 60–80% Time: 18 days	<ul style="list-style-type: none"> Water content decreases Amino acids decrease 	Reducing sugar levels increase	–	–	Total phenols decrease	Sailah and Miladulhaq (2021)
	Fresh garlic	Temperature: (1) 60 °C (A1) (2) 70 °C (A2) (3) 80 °C (A3) RH: -Time: (1) 7 days (B1) (2) 14 days (B2) (1) (3) 21 days (B3)	<ul style="list-style-type: none"> The lowest moisture content is at 80 °C usage for 21 days The higher temperature and aging period, the higher protein content 	Increased reducing sugar	–	The higher temperature and longer the aging time, antioxidant levels increase	Increased polyphenol levels	Herlina et al. (2019)
	Fresh garlic	Temperature: (1) 50 °C (2) 60 °C (3) 70 °C (4) 80 °C (5) 90 °C Rh: (1) 50% (2) 60% (3) 70% (4) 80% (5) 90% Time: (1) 120 h (2) 150 h (3) 180 h (4) 210 h (1) (5) 240 h	–	–	<ul style="list-style-type: none"> Enzymatic activity (Allinae, γ-GTP, and γ-glutamyl-cysteine synthetase) decreases The compound alliin increases until the 180th hour before continue to decrease. The γ-glutamyl cysteine compound increases slowly until the 210th hour before finally decreasing. SAC increase up to 210 h of aging before then decreasing. RH affects γ-GTP activity compared to other enzymes (80% most optimal) Temperature of 70 °C produces the best content of alliin, SAC, and 	–	–	Liu et al. (2022)

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Table 4 (continued)

No	Type	Aging Conditions	Physical and Nutritional Composition	Polysaccharides and Maillard Products	Organosulfur	Antioxidant	Other compounds	Reference
					γ -glutamyl cysteine among other temperatures			

Describe: DPPH (2,2-difenil-1-pikrilhidrazil), FRAP (Ferric Reducing Antioxidant Power); IC50 (requirement concentration level of antioxidant to decrease 50% of DPPH radical activity); GAE (Garlic Acid Equivalent); GAEAC (Garlic Acid Equivalent Antioxidant Capacity).

Table 5
Endophytic Microorganisms and their Role in Black Garlic Fermentation.

Microorganism	Roles	References
<i>Saccharomyces cerevisiae</i>	Production of bioactive	Jung et al. (2011)
<i>Bacillus subtilis</i>	Antihypertensive activity and volative compound and organic acid, production of bioactive	Park et al. (2016)
<i>Bacillus licheniformis</i>		
<i>Bacillus sp</i>		
<i>Lactic plantarum</i>	Inhibition of pathogenic	Bernbom et al. (2009)
<i>Lactobacillus plantarum</i>	Inhibition of pathogenic	Bernbom et al. (2009)
<i>Weissella koreensis</i>	Immunity enhancement	Wang et al. (2011)

6.2. Aging period

Aging time is another factor that is no less important than temperature and humidity. Based on the results of the study attached in Table 5 above, the aging time shows an effect on the psychochemical and functional changes of black garlic (Azhar et al., 2021; Dewi, 2018; Herlina et al., 2019; Kang, 2016b; Liu et al., 2022; Nakagawa et al., 2020; Nelwida et al., 2019; Putri et al., 2020; Sailah and Miladulhaq, 2021; Thalia et al., 2020; Toledano Medina et al., 2016; Yu et al., 2020). Semakin lama waktu *aging* yang digunakan, Kadar air dan pH tampak mengalami penurunan, berbanding terbalik dengan intensitas pencoklatan dan rasa manis yang semakin meningkat karena besarnya kadar monosakarida. Penurunan kadar air hingga hari ke-90 secara konsisten mempermudah karbohidrat untuk terdegradasi dan menciptakan substrat bagi reaksi maillard (Dewi, 2018).

Nelwida et al. (2019) stated that the increase in reducing sugar continuously until the 45th day was caused by consistent heating, resulting in the hydrolysis of polysaccharides. The reducing sugar produced by hydrolysis is greater than as a substrate for the Maillard reaction (Sailah and Miladulhaq, 2021; Zhang et al., 2015). It also creates a sweet taste of black garlic at the end of the production process (Dewi, 2018). Aging carried out on the 40th day at 80 °C also had a more intense color intensity than the 30th day sample at 60 °C which indicated the occurrence of non-enzymatic browning consistently (Thalia et al., 2020). In addition, maillard products such as 5-HMF and melanoidin also increased (0.5 mg/g within 7 days) on the 28th (last) day of aging compared to the 0, 7, 14, and 21 days (Nakagawa et al., 2020).

Differences in the aging process also affect the level of organosulfur levels. There was a decrease in alliin levels by 85.9%, a decrease in the activity of the enzymes allinase, -GTP, and -glutamyl-cysteine synthetase, as well as an increase in the content of SAC and -glutamyl cysteine (γ -GC) at the end of the aging process (407 h). Based on the single-factor experiment, it was concluded that the optimal time for aging at 70 °C and 80% humidity is 180 h (7.5 days), while at 80 °C and 90% humidity is 210 h (8–9 days). day) (Liu et al., 2022). The capacity of DPPH scavenging, TEAC, and antioxidant activity against SOD as an antioxidant indicator of black garlic also increased until day 35. Apart from antioxidants. The highest bacteriostatic ability of black garlic against *B. subtilis* and *S. aureus* was shown on the 35th day of aging (Chang and Jang, 2021).

Lee et al. (2011) in a study of black garlics that were processed at 70 °C concluded that the longer the aging was carried out, the better the quality of the black garlic produced. In addition, the aging time can also be shortened by balancing other factors such as high temperature settings (>85 °C) or the application of pretreatment which can trigger polysaccharide degradation and other psychochemical changes in black garlic (Li et al., 2015; Liang et al., 2015; Karina L. Ríos-Ríos et al., 2019). On the other hand, aging that is carried out for too long can also result in excessive hydrolysis of polysaccharides resulting in a very soft texture of black garlic due to the high humidity of the aging environment, thus encouraging a rapid rate of polysaccharide degradation (Kang, 2016b; Putri et al., 2020).

6.3. Microorganism activity and antibacterial activity

Endophytic microorganisms in addition to having a role as biocontrol can also stimulate plant growth and support antimicrobial properties against pathogenic bacteria (Resti et al., 2017). Some researchers say that black garlic is processed through spontaneous fermentation due to the activity of endophytic microorganisms from garlic (Lu et al., 2018).

Several studies have found the role of microorganisms in black garlic, especially finding the role of endophytic microorganisms, especially from fungal and bacterial endophytes (Table 5). Endophytic microbes that were successfully isolated from black garlic were from the genus *Erwinia*, *Pseudomonas*, *Xanthomonas*, *Agrobacteria*, *Ralstonia*, *Xylophilus*, *Pantoea*, *Acidovorax*, *Burkholderia*, *Coryneform*, and *Streptomyces*. These endophytic microorganisms produce organic acid compounds such as tartaric acid, succinic acid, malic acid and acetic acid from the glucose fermentation process (Lestari et al., 2021).

A number of endophytes that have been isolated originate from fungal and bacterial endophytes. This endophyte plays a role in fermentation by producing the characteristic taste and aroma of black garlic. Endophytes can speed up the processing of black garlic, improve the taste and functional substances and extend the storage period (Ramesh et al., 2014).

In addition, some endophytic groups produce lipopeptides, peptides, surfactants and fengysin which can inhibit the growth of pathogens. The fermentation process breaks down the glucose content in black garlic, this glucose then becomes a carbon source for the growth of bacterial and fungal endophytes. The endophytes that were isolated from black garlic in this study were *B. subtilis*, *B. methylotrophicus*, and *B. amyloliquefaciens* (Lu, et al., 2018).

Until now, black garlic production still prioritizes aging factors based on the effect on enzymatic and non-enzymatic browning reactions including a combination of temperature, environmental humidity, time, and pretreatment. Although it is still quite limited, several studies have identified the presence of endophytic bacteria from the genus *Bacillus* that can survive aging (Lestari et al., 2021; Qiu et al., 2021; Lu et al., 2018). Temperature, water content, and water activity (*Aw*) are important components for microbial growth and chemical reactions (González-Ramírez et al., 2022). *Bacillus* is a genus of bacteria that mostly includes rod-shaped gram-positive bacteria, belongs to facultative aerobic and anaerobic bacteria and is the most abundant endophytic bacteria in plants (de Melo et al., 2021).

According to observations [Yani et al. \(2019\)](#) endophytic bacteria *Bacillus* sp in Andalas plant has an inhibitory power against pathogenic bacteria *E. coli* and *S. aureus* at 37 °C and pH 7.0 (neutral). However, the growth of *Bacillus subtilis* and *Bacillus licheniformis* was well identified at a temperature of 40–50 °C and *Bacillus methylotrophicus* and *Bacillus amyloliquefaciens* at a temperature of 30–40 °C with a pH of 5–9 ([Z. Qiu et al., 2021](#)). This indicated that the four strains had good resistance to temperature and pH conditions that were different from their optimal growth temperatures. *B. licheniformis* resistance tended to be better than the other three strains. *B. licheniformis* had a D50 °C value of 20.92 min and was greater than *B. subtilis* with a D50 °C value of 10.59 min. The smaller the D value obtained, the faster the time to inactivate the bacteria ([Adeyinka and Dapo, 2020](#)). [Berendsen et al. \(2016\)](#) his study also added that there is heat resistance of all strains of *Bacillus licheniformis* and *Bacillus amyloliquefaciens* up to a temperature of 57.7 °C. This resistance to high temperatures is thought to be influenced by high humidity settings during aging.

The presence of *Bacillus* sp has been shown to have a strong influence in increasing the total acid and the amount of reducing sugar through the fermentation process in black garlic ([Lestari et al., 2021](#); [Lu et al., 2018](#)). Research by ([Qiu et al., 2019](#)) explained the presence of inulinase activity in 4 strains of *Bacillus* sp with the highest production by *Bacillus subtilis* at 60 h of aging. Although inulinase activity can only last up to 72 h by *B. licheniformis* before decreasing, inulinase which is an inulin hydrolyzing enzyme actively converts inulin-type fructan preparations (mostly found in garlic) into fructose or fructo-oligosaccharides ([Saryono et al., 2016](#)). These data strengthen the alleged involvement of endophytic microorganisms in degrading polysaccharides during aging.

Garlic initially also had the endophytic fungus *Trichoderma brevicompactum* which was strong antifungal against *R. solani* with an EC50 of 0.25 g/mL-1, then against *Botrytis cinerea*, *Fusarium oxysporum*, *Colletotrichum ampelinum*, and *Colletotrichum lindemuthianum* ([Shentu et al., 2014](#)). However, until now there has been no research that has succeeded in identifying the activity of molds during aging. This could be

due to the incompatibility of the mold growth environment with aging conditions.

Trichoderma brevicompactum was found in black garlic as a fungal endophyte isolate that was successfully isolated. *Trichoderma brevicompactum* which has the ability to inhibit the activity of *Rhizoctonia solani* and *Botrytis cinerea* as pathogenic fungi in black garlic ([Lu, et al., 2018](#)). *Trichoderma brevicompactum* shows strong antifungal activity in black garlic. *Trichoderma* receives less attention because in general it is a species of soil-borne fungus that plays a role in the ecology and rhizosphere of plants. However, there is an active compound called trichodermin which is a compound that inhibits the growth and activity of pathogenic fungi. So that the endophytic *T. brevicompactum* has great potential as an antifungal by producing trichodermin compounds ([Shentu et al., 2014](#)).

Research identified *Trichoderma* fungus in black garlic. Research has been carried out using other fungal groups, but *Trichoderma* is the most effective isolate, even when used individually *Trichoderma* even suppresses rot in garlic ([Shentu et al., 2014](#)). Even though *Trichoderma* is considered an antagonistic fungus, in black garlic, this endophyte plays a very effective role in black garlic.

Currently, there is no research that explains the dangers and risks that could occur regarding the involvement of *Trichoderma* isolates in black garlic. This research ([Elshahawy et al., 2019](#)) shows another advantage caused by *Trichoderma* in black garlic, that it can increase the growth of garlic more significantly.

As shown in [Table 6](#), black garlic has antimicrobial effects against several pathogenic bacteria such as *E. coli*, *S. aureus*, *S. typhi*, and *P. aeruginosa* ([Chang and Jang, 2021](#); [Thalia et al., 2020](#)). Inhibition of *S. aureus* in both studies had lower antimicrobial inhibition compared to other strains. This is because in the early aging temperature range (± 40 °C) the availability of allicin compounds is still quite large. Allicin is a fat-soluble organosulfur compound so that it is easier to react to gram-negative bacteria which have a lipopolysaccharide cell wall layer structure that is thicker than peptidoglycan. On the other hand,

Table 6
Endophytic microorganisms and antimicrobial activity of black garlic.

No.	Type	Table 6	Types of Endophytic Microorganisms	Antimicrobial Activity	Reference
1	Black garlic	(1) Potato dextrose agar (PDA) (2) Beef extract peptone agar (3) Gauze media	(1) <i>Bacillus altitudinis</i> sp. (2) <i>Bacillus methylotrophicus</i> sp. (3) <i>Bacillus aryabhatai</i> sp. (4) <i>Bacillus siamensis</i> sp. (5) <i>Bacillus subtilis</i> sp. (6) <i>Bacillus pumilus</i> sp. (7) <i>Bacillus macroides</i> sp.	–	(Qiu et al., 2018)
2	Black garlic	(1) Potato dextrose agar (PDA) (2) Nutrient agar (NA)	(1) <i>Bacillus subtilis</i> (2) <i>Bacillus methylotrophicus</i> (3) <i>Bacillus amyloliquefaciens</i>	–	Lestari et al. (2021)
3	Black garlic	(1) <i>Beef extract peptone agar</i> (BPA) (2) <i>Beef extract peptone liquid</i> (BPL) (3) <i>Trypticase soy broth</i> (TSB) (4) LB broth (5) <i>Peptone water</i> (6) <i>Plate count agar</i> (PCA)	(1) <i>Bacillus subtilis</i> (2) <i>Bacillus licheniformis</i> (3) <i>Bacillus methylotrophicus</i> (4) <i>Bacillus amyloliquefaciens</i>	–	(Qiu et al., 2021a)
4	Black garlic	(1) <i>Plate count agar</i> (PCA)	–	(Lowest; highest) (1) <i>Escherichia coli</i> : 11.0 mm (60 °C, 40 days); 12.8 mm (70 °C, 40 days) (2) <i>Staphylococcus aureus</i> : 10.5 mm (60 °C, 30 days); 12.5 mm (80 °C, 30 days) (3) <i>Salmonella typhi</i> : 12.1 mm (60 °C, 40 days); 13.1 (70 °C, 40 days)	Thalia et al. (2020)
5	Black garlic	(1) <i>Nutrient broth</i> (NB) (2) <i>Mueller hinton agar</i> (MHA) medium	–	(1) <i>Staphylococcus aureus</i> : 13.33 mm (5th day); 23.33 mm (35th day) (2) <i>Escherichia coli</i> : 88.67 mm (15th, 20th, 25th, and 40th days); 9.67 mm (35th day) (3) <i>Pseudomonas aeruginosa</i> : 10.33 mm (5th day); 12.67 mm (35th day)	Chang and Jang (2021)

gram-positive bacteria are more resistant because they contain a polar peptidoglycan layer so they are not able to react to fat-soluble compounds as well as gram-negative bacteria (Chang and Jang, 2021; Handayani et al., 2019; Thalia et al., 2020).

Bacillus belongs to the endophytic bacteria which helps in the fermentation of black garlic polysaccharides. However, the concentration of these bacteria decreased with increasing organosulfur and 5-hydroxymethyl furfural (Qiu et al., 2019). Several researchers conducted tests on other lactic acid bacteria such as *Lactobacillus* strains added to the manufacture of black garlic (non-spontaneous fermentation). Samples of fermented black garlic *L. bulgaricus*, *L. plantarum*, *L. rhamnosus*, and *L. fermentum* had total sugar, reducing sugar, protein, and also greater functional ability and lower water content and pH than without the addition of lactic acid bacteria (Ma et al., 2021; Ro et al., 2022; Si et al., 2019).

The addition of lactic acid bacteria increases the production of several organic acid products such as formic acid, acetic acid, propionic acid, and butyric acid through the glycolysis process and suppresses the growth of 5-HMF to prevent excessive 5-HMF levels (Nakagawa et al., 2020; Ma et al., 2021). *Lactobacillus* sp grows optimally at a temperature of 30 °C and pH 6. This type of bacteria is able to stimulate intestinal health, reduce the potential for absorption of carcinogenic substances, reduce the risk of colorectal cancer, and has anti-inflammatory properties so that it is widely used in the food fermentation process (Hadinia et al., 2022). The high levels of antioxidant compounds including organosulfur compounds (such as SAC) and polysaccharides and their derivatives in fermented black garlic not only increase antioxidant activity but are also good for the balance of intestinal microbiota and gestational diabetes mellitus (Ro et al., 2022; Si et al., 2019).

Moreover, it is the fermentation process that can maintain antioxidant activity because the fermentation process shows that fermented garlic has reduced water content, but the levels of crude protein, crude fiber, crude ash and carbohydrates increase significantly after fermentation. In addition, the mineral content also increases. Antioxidant activity is also higher after fermentation, following the references (Tahir et al., 2022) increasing antioxidants with total phenolic content and total flavonoid content increasing after black garlic is fermented. Fermentation by microbes also makes the pH of black garlic low and this can maintain the components, especially antioxidants. Research shows (Z. Qiu et al., 2020) that black garlic is superior to unfermented garlic. However, special attention needs to be paid to the initial treatment, temperature and humidity during processing so that microbial interactions can be as desired for optimal effectiveness.

Research by (Jung et al., 2011) found stronger in vitro antioxidant and hepatoprotective and nephroprotective activity in fermented black onions with the yeast *Saccharomyces cerevisiae*. What's more, garlic fermented with *Bacillus subtilis* is known to reduce blood pressure or hypertension (Park et al., 2016). *Bacillus* sp. Species can increase the sensory and bioactive properties found in black garlic. Bioactive substances that are useful as antioxidants can be produced through fermentation with several strains (Lu, et al., 2018) research results show that the fermentation process can produce bioactive substances and bioactivity that is superior to unfermented garlic. Moreover, Microbial fermentation of black garlic produces glucose and mannose which have strong antioxidant activity and increase immunological activity. So it can be seen that fermentation is considered an effective way to maintain and increase the antioxidant activity of black garlic. Given that fermentation is a precise and effective method compared to other methods.

6.4. Pretreatment

Pretreatment is a process step that is carried out on the basic ingredients, namely garlic using various existing methods (generally physically and chemically) by utilizing aging factors such as temperature as a step to optimize organoleptic, psychochemical, and biological

activity characteristics in black garlic (Kim et al., 2013). The application of Pretreatment in the manufacture of black garlic also varies, either through heating, cooling, and so on.

Each pretreatment in Table 7 shows the results of different characteristics of black garlic so that it can be seen that pretreatment has an influence as other factors. Freezing pretreatment is an alternative method that is widely applied to shorten the aging process of black garlic through cell destruction due to freezing injury. Based on the studies that have been carried out, freezing pretreatment can produce black garlic with reducing sugar content, degree of browning, 5-HMF, total phenolic, and SAC along with other organosulfur better than black garlic without freezing treatment (Kandemirli et al., 2020; Li et al., 2015; Yuan et al., 2018).

Samples with freezing treatment for 30 h had reducing sugar content 1.7 times higher than ordinary black garlic (control sample). Freezing treatment helps in destroying the structure of the garlic cell membrane making it easier for substrates and enzymes to react, creating reactions such as fructan hydrolysis so that fructose levels increase up to 508.11% (Yuan et al., 2018). The Maillard reaction also seems to run faster which triggers an increase in 5-HMF and melanoidin compounds to reach optimal values on the 22nd day of aging. However, excessive 5-HMF content causes a bitter taste in the product. In addition, the fast Maillard reaction caused the amino acid content to decrease immediately after the 10th day to 50.97% (Li et al., 2015).

The freezing process before aging encourages the formation of ice crystals in the cells which then results in the rupture of the vacuole and loss of turgor pressure. This causes the cell structure to become damaged, forming large spaces in the damaged tissue. Through damaged cell membranes, enzymes and substrates can react more easily and longer because it creates conditions that are still feasible with high humidity for a longer time even though using high temperatures (Yudhistira et al., 2022). Park et al. (2014) proved an increase in the activity of the γ -glutamyl transpeptidase (GGT) enzyme at low temperature storage, resulting in a high SAC content. Therefore, the formation of SAC and other organosulfur compounds can be up to 4.6 times higher than that of ordinary black garlic without any physical deviations (Chen et al., 2020). The results of the analysis of DPPH, FRAP, total phenolic, total flavonoid, and large antioxidant capacity also represent high antioxidant, anticarcinogenic, and high biological activity in black garlic by freezing pretreatment (Kandemirli et al., 2020).

Drying pretreatment is a basic method that is effective in preventing the growth of pathogenic and spoilage microbes through evaporation of 90% of the water content in food ingredients. Drying techniques that have been widely applied in the food industry are diverse, including hot air drying, infrared drying, microwave drying, vacuum drying, and vacuum freeze drying (Feng et al., 2021). The use of the Convective drying pretreatment technique resulted in a product with high humidity producing a psychochemical value that was almost the same as that of black garlic in general on the 4th day of aging, but did not have a quality value that was bad enough because it had a high level of spiciness that was close to fresh garlic (Ríos-Ríos et al., 2018). Therefore, this method is not considered to be well applied as a pretreatment of black garlic. Another opinion revealed that the use of drying pretreatment can improve the quality of black garlic if drying is only done to evaporate the water content on the surface until the garlic reaches the water content limit of 70–72% (Hu et al., 2022).

In addition to drying, other thermal methods such as steaming pretreatment showed good results in increasing the bioactive compounds, physical characteristics, and antioxidants of black garlic (Karnjanapratum et al., 2021). Steaming can increase the degree of browning and produce a darker color as the steaming time increases. Like blanching, balanced steaming temperature and humidity conditions encourage damage to the cell wall structure which continues to the polysaccharide degradation stage and the Maillard reaction (Quayson et al., 2021). The rate of browning is also faster on day 4 because the polyphenol oxidase enzyme that plays a role in enzymatic browning is still active (Fante and

Table 7
Application of various Pretreatment Methods on Aging Black Garlic.

No.	Type	Pretreatment Method	Results	Reference
	Fresh garlic	High Hydrostatic Pressure (HHP)	<ul style="list-style-type: none"> • HHP in acidic conditions is more effective in lowering pH • Textures are softer than controls • Improves browning time • Maintains antioxidant activity • Helps suppress the growth of microbes of <i>S. aureus</i>, <i>L. innocua</i>, <i>S. mutans</i>, <i>S. enterica</i>, and <i>P. aeruginosa</i> 	Kim et al. (2014)
	Laiwu hybrid garlic (70% moisture content, 17,969 mg/g reducing sugar, 4689 mg/g amino-nitrogen, and 3206 mg/g total phenols)	Freezing pretreatment (30 h)	<ul style="list-style-type: none"> • Increases the degree of browning. • Increases the content of reducing sugars. • Lower amino acid levels compared to controls. • Increases total phenolic. • Increased production of 5-HMF. 	Li et al. (2015)
	Mexican purple garlic bulbs	Drying pretreatment (temperature: 70 °C; RH: 9%; 4 days)	<ul style="list-style-type: none"> • Promotes a decrease in the moisture content of black garlics. • Resulting in a lower pH reduction compared to the control (the resulting pH is higher than the control) • Pushing down Aw rather than control. • Samples with drying pretreatment had a higher level of spiciness than controls. • Lower fructose than control • Increases 5-HMF production but not as large as black garlics in general. 	
	Fresh garlic	Freezing pretreatment (temperature: 50 °C; RH: 80%; 90 days)	<ul style="list-style-type: none"> • Fructant content decreased by 84.79% • Fructose levels increased by 508.11% • The content of amadori and heyns 	Yuan et al. (2018)

Table 7 (continued)

No.	Type	Pretreatment Method	Results	Reference
	Fresh garlic	High Hydrostatic Pressure (HHP) (temperature: 40 °C; RH: 85%; 10 days)	<p>compounds increases (amadori concentration > heyns concentration)</p> <ul style="list-style-type: none"> • pH: ±4 • The largest γ-GTP activity is at a pressure of 300 MPa and the smallest at 100 MPa (HHP increases γ-GTP activity) • The largest SAC content is at a pressure of 300 MPa and the smallest at 100 MPa • The longer the pretreatment time, the greater the SAC content of black garlics. • The greatest browning intensity is at a pressure of 200 MPa and the smallest at 100 MPa • The longer the pretreatment time, the smaller the intensity of browning black garlics. 	Chen et al. (2020)
	Fresh garlic	Freezing pretreatment (pretreatment: 16 °C, 30 h Aging: 60 °C, 35 days)	<ul style="list-style-type: none"> • DPPH: frozen samples are larger than non-frozen • Total phenolics: frozen samples are larger than non-frozen • Total antioxidant capacity: frozen samples are larger than non-frozen • Total flavonoids: frozen samples are larger than non-frozen • FRAP: frozen sample equal to/ and or greater than non-frozen • HMF: frozen samples are larger than non-frozen 	Kandemirli et al. (2020)
	Fresh garlic	Steaming pretreatment (temperature: 60 °C; 18 days)	<ul style="list-style-type: none"> • Lowering the degree of browning of black garlics • Produces a soft black garlic texture • Total phenolic increases 	Karnjanapratum et al. (2021)

(continued on next page)

Table 7 (continued)

No.	Type	Pretreatment Method	Results	Reference
	Fresh garlic	High Hydrostatic Pressure (HHP)	<ul style="list-style-type: none"> Total flavonoids increase DPPH increases with time FRAP increases with time Cells are plasmolyzed so that it encourages the reaction to run faster Fructant content decreases to 50.95% w/w Sucrose content decreases to 12.22 mg/g Fructose content increases to 3.85 mg/g Effectively maintains the activity of feh enzymes The intensity of browning with HHP increases to 1.60 Lowers the pH of black garlicks until it reaches 4.06 Lowers the moisture content of black garlicks to reach 54.87% Increased reducing sugar content Total amino acids decrease slightly Increased HMF production by 1007.33 µg/g Increased total phenolic by 943.56 mg/g Slightly lowered alliin levels by 279.44 µg/g Increases SAC levels to 1033.92 µg/g Garlic with HHP has a quality value of 88.63 	Li et al. (2020a)
	Garlic (purple bulbs)	Ohmic heating pretreatment (pretreatment: 110–130 V; 60–80 °C) (aging: 70 °C; 94%; 30 days)	<ul style="list-style-type: none"> Slightly lowers the level of spiciness It has a higher total of polyphenols than the control sample. Increases the antioxidant capacity of black garlicks (DPPH: 48.9%) Increases HMF content by 114.3 mg/100g 	Ríos-Ríos et al. (2021)

Table 7 (continued)

No.	Type	Pretreatment Method	Results	Reference
	Fresh garlic	High hydrostatic pressure (HHP)	<ul style="list-style-type: none"> Fructose levels were lower than controls. Glucose levels were lower than controls. Sucrose levels were lower than controls. Lowers fructant levels to 161.7 mg/100g. Improves color density, color polymer, and melanoidin browning index The best HHP treatment is at a pressure of 400 MPa 	Zhao et al. (2019)

Noreña, 2012). Antioxidant activity in DPPH and FRAP parameters also showed an increase until day 18 along with the increase in antioxidant compounds and other biological activities (Karnjanapratum et al., 2021).

Ohmic heating is basically done by passing an electric field on the material which aims to heat food quickly and evenly. This method allows heating with an accurate temperature that affects the induction of membrane permeability, increasing the diffusion of molecules in vegetable tissues, drying, pasteurization, to the extraction of vegetable compounds (Pereira et al., 2021). The use of ohmic heating is able to shorten the production time from 30 days to 12 days with similar characteristics. Although it has good organoleptic value, total phenolic, and reducing sugar, it is necessary to pay attention to the ohmic heating setting so as not to produce 5-HMF and a high level of spiciness because it can cause bitterness in the product (Ríos-Ríos et al., 2021).

High hydrostatic pressure (HHP) pretreatment is a non-thermal method that is able to change the cell structure of microorganisms which leads to cell death due to inactivation of enzymes or modification of membrane permeability. Therefore, HHP is generally used to sterilize microorganisms, inactivate enzymes, change protein properties and form a gel structure without changing the nutritional content of the food (Kim et al., 2013). The HHP method accelerates the occurrence of the Maillard reaction so that the sample appears to start browning on day 2 (faster than the control). Although the inactivation of the FEH enzyme became more rapid, the rapid increase in fructose levels was supported by the degradation of polysaccharides due to cell changes at high pressure (Li et al., 2020).

Overall, HHP maintains the quality of black garlic so that the sample has the same taste characteristics as black garlic in general. HMF levels only increased at the beginning of the application of pressure then stabilized on day 18. This makes the control HMF levels greater than the HHP samples (Li et al., 2015). Zhao et al. (2019) also added that the greater the pressure used, HHP was able to suppress the increase and stabilize the melanoidin content which had an impact on the color density, color polymer, and browning index of the melanoid extract with the best pressure at 400 MPa. The HHP method also helps in increasing the enzymatic activity of -GTP along with increasing temperature and pressure before being inactivated by breaking non-covalent bonds such as hydrogen bonds. This then resulted in black garlic with SAC content up to 7 times higher than black garlic without HHP treatment and known to be higher than black garlic products with freezing treatment (Y. T. Chen et al., 2020; S. H. Park et al., 2014).

7. Functional properties

Organosulfur compounds in black garlic such as S-allyl cysteine have diverse biological activities that bring a good impact to human's health (Qiu et al., 2020). Thermal processing changes physicochemical and stimulate the presence of differences functional properties of black garlic compared to fresh garlic.

7.1. Antioxidant

A main reason of garlic application in traditional medicines is because garlic contains high-antioxidant (Jeong et al., 2016). Antioxidants effectively inhibit oxidation through binding with highly reactive free radical compounds. This binding can also inhibit the cycle of adverse effects of free radicals which in turn can cause damage to a cell or tissue and result in the emergence of various diseases both autoimmune diseases, degenerative, to cancer (Azhar et al., 2021). Although allicin levels in black garlic is limited, some other stable organosulfur compounds such as S-allyl cysteine (SAC), s-allyl mercapto cysteine (SAMC), and allyl sulfide which formed during thermal processing have a high antioxidant activity (Locatelli et al., 2017).

Several studies on antioxidant properties in black garlic using DPPH radical scavenging, ABTS, Ferric Reducing Antioxidant Power (FRAP), and reducing power assays analysis shown increase during thermal treatment. It can be interpreted that enzymatic and non-enzymatic reactions affect antioxidant levels of black garlic (Chen et al., 2020; Choi et al., 2014; Jeong et al., 2016; Lu et al., 2017; Medina et al., 2019). Black garlic contains antioxidant activity of 5–7 times greater than garlic caused by increased content of polyphenols and S-Allyl cysteine (SAC). The increase was triggered by a non-enzymatic browning reaction during aging (Ding et al., 2021).

Kimura et al. (2017) revealed growth of antioxidant about 10 times higher according to superoxide dismutase and scavenging activity against hydrogen peroxide in black garlic samples fermented for 40 days (60–70 °C; RH 85–95%) compared to fresh garlic. The higher antioxidant capacity mostly caused by the formation of SACS during aging. SAC is capable to destroy superoxide anion radicals ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), hydroxyl radicals ($\bullet OH$), peroxyxynitrite anions ($ONOO^-$), hydroxyl and peroxy radical forms at certain concentrations. The antioxidant power of the SAC will be reduced when the SAC loses allyl group which indicates the importance of allyl group in supporting scavenging activity (Colín-González et al., 2012; García-Villalón et al., 2016).

Phenol oxidation and polysaccharide depolymerization occur during the thermal incubation process. Heat treatment will accelerate the degradation of high molecular weight polysaccharides into compounds with lower molecular weight, namely oligosaccharides and monosaccharides. Then conversion will occur into water-soluble bioactive compounds such as S-allylcysteine, tetrahydro-b-carbolines, alkaloids, polyphenols and flavonoid-like compounds. This compound has strong activity as an antioxidant and can be useful as an anti-tumor, anti-cancer, anti-allergic, and hypolipidemic. This compound will increase during the thermal process in black garlic. That's why you have to pay attention to the optimal time for the incubation process. because the antioxidant properties of red black onions are optimal on day 21 of the incubation period. This also explains why red garlic has high antioxidant activity despite heat treatment due to the conversion of water-soluble bioactive compounds (Karnjanapratum et al., 2021).

The antioxidant compounds contained in black garlic are maintained and even have a higher content after processing. The high temperature and humidity that occurs results in higher polysaccharide content, higher protein, phenolic compounds and organic sulfur as well as higher melanoidin. The antioxidant properties of black garlic are related to the polyphenol content. Polyphenolic compounds are sensitive to heat. However, the amount of polyphenols increases 3x compared to whole garlic. as well as an increase in the activity of polyphenols and

flavonoids that occur during the aging process (Lu et al., 2017).

Apart from that, there is a lactic acid compound which is formed from the fermentation of black garlic under hot and humid temperature conditions. This lactic acid also has strong antioxidant activity, which is why antioxidant activity is found in black garlic even though it is processed at high temperatures. Moreover, high temperature conditions in the process are closely related to the Maillard reaction and have anti-oxidant activity (Queiroz et al., 2009).

7.2. Anti-inflammatory

Inflammation primarily been use in responding injuries, infections, and toxins that infected body system as a protection from unknown external substances most likely virus and bacteria. However, excessive inflammation can cause damage that leads to various diseases such as cardiovascular disease, cancer, neurogenerative disorders, and various other chronic disorders (Kim et al., 2017; Tran et al., 2019). Some components in black garlic include pyruvate, 5-hydroxymethyl furfural (5-HMF) and organosulfur components (DAS, DADS, DATS, S-allyl cysteine, alliin, allicin, and ajoene) have anti-inflammatory effects that are in line with antioxidant activity (Ryu and Kang, 2017).

When abundant pyruvate combined with allicin, it significantly reduce the regeneration ability of ROS and H_2O_2 , and also inhibit the production of NO and PGE_2 (Jeong et al., 2016). Another research about Indonesian black garlic (IBG) on macrophage cells 264.7 explained the decrease in NO production as an indicator of pro-inflammatory cytokines and impact on the body's immune power. The anti-inflammatory mechanism of IBG consists of 2 possibilities, namely inhibition of pro-inflammatory mediators (Toll-like receptor (TLR-4)) associated with inhibition of nuclear factor- κB (NF- κB) activity and also antioxidant activity that suppresses the production of NO and ROS (Pratidina et al., 2021).

7.3. Anti-cancer

Bioactive components in functional foods have a strong power in treating inflammation and carcinogenic substances as well as potential as a chemo-preventive agent (Aghajanpour et al., 2017). Studies using In vivo and In vitro method on black garlic have proved the presence of good anticancer activity. Dong et al. (2014) found a strong antioxidant and anticancer activity from black garlic extract against cancer cell growth in HT29 intestinal cells by inhibiting cell growth, induction of apoptosis, and restraining cell cycle in HT29 cells.

Park et al. (2014) research on leukemia cancer cells also present a positive response due to the anticancer ability of black garlic extract. S-allyl cysteine (SAC) on black garlic extract (temperature 65–80 °C; RH 70–80%) identified has an anticancer activity against t47d breast cancer cells against several cancer models consisting of ovarian carcinoma, prostate, and hepatocellular (Permatasari et al., 2020). Another sulfur compounds such as SAMC, DAS, and DADS similarly have a high scavenging activity against free radical compounds and destroy ROS directly. ROS involved in malignant gastric pathogenesis but can be combated using SOD (superoxide dismutase). Black garlic extract could affects the increase of SOD and GSH-Px in gastric cancer cells by in vitro method on animals (Wang et al., 2012).

8. Conclusion and future perspectives

Black garlic was originally formed as a solution to reduce the unpleasant characteristics in fresh garlic. Instead of forming consume-friendly organoleptic, aging process also has proven in producing functional properties and health benefit. Several researches have shown organosulfur as the most widely related compounds to various biological activities such as antioxidant, anti-inflammatory and anticancer which are useful in maintaining and curing lot of diseases. Not only as a functional food, black garlic has been applied in manufacture industry

mostly at food industry, pharmaceuticals, and medical purposes.

Thus far, research related to black garlic still increasingly widespread both from determining the best aging preparation (degrees, humidity, and time), pretreatment application, and another related reaction most likely lactic bacteria's role, especially due to the organosulfur content to obtain the best standards to maximize production on an industrial scale. Most of the functions of black garlic include antioxidant, anticancer, Cardiovascular protective, and as an immunostimulant is closely associated to the presence of organosulfur compounds, mainly SAC and SAMC. Although it does not have much research as much as another functional food, considering to their benefit and positive results in most of in vitro and in vivo researches, black garlic could be the best alternative solutions from functional food group to increase bio-medicine availability supported by further studies in it organosulfur and another bioactive compound.

CRedit authorship contribution statement

Gemilang Lara Utama: Conceptualization, Supervision, Writing – review & editing, Validation. **Zahida Rahmi:** Investigation, Data curation, Visualization, writing. **Meli Puspita Sari:** Writing – review & editing. **In-in Hanidah:** Supervision, Writing – review & editing, The authors have given final approval for the version to be published, and all authors read and approved the final manuscript.

Declaration of competing interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Data availability

Data will be made available on request.

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