1 **Physicochemical changes and sensorial properties during black garlic elaboration: a**

2 **review**

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5 **Karina L. Ríos-Ríos, Antonia Montilla*, Agustín Olano, Mar Villamiel**

6 *Instituto de Investigación en Ciencias de la Alimentación (CIAL), (CSIC-UAM), C/Nicolás

7 Cabrera, 9, Campus de la Universidad Autónoma de Madrid, 28049 Madrid, Spain

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14 *Author to whom correspondence should be addressed

15 E-mail address: a.montilla@csic.es;

16 Tel: +34 910017952

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18 ABSTRACT

19 *Background:* Black garlic (BG) is produced by the heat treatment of fresh garlic (FG) bulbs

20 under controlled conditions of high temperature and high relative humidity for long periods

21 of time without any additional treatment or additives. During this thermal process, changes

22 in the chemical and physicochemical composition of BG are produced to a in very different

23 magnitude, mainly affecting volatile sulfur compounds, free amino acids, polyphenols and

24 carbohydrates

25 *Scope and approach:* By analysing these changes, studies about the mechanism involved in

26 the BG elaboration have been conducted. Recent scientific findings indicate that the Maillard

27 reaction (MR) is responsible for BG transformation, leaving behind the hypothesis of

28 spontaneous fermentation.

29 *Key findings and conclusions*: We summarize the knowledge of the main chemical and

30 physicochemical changes that take place during the BG obtainment paying special attention

31 to non-enzymatic browning reactions. Thus, the trends in BG research are related to the

32 usefulness of MR compounds as indicators of quality for controlling the non-enzymatic

33 process of BG as well as the evidence of the important role of the conditions of moisture and

34 temperature required for the industrial production of BG.

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36 *Keywords*: Thermal processing, non-enzymatic browning reactions, Maillard reaction,

37 chemical compounds, chemical changes.

38

1. Introduction

Garlic (*Allium sativum* L.) has been widely used for millennia not only for cooking but also in traditional medicine to treat a wide range of disorders (Corzo-Martínez, Corzo & Villamiel, 2007; Kimura, Tung, Pan, Su, Lai & Cheng, 2017; Santhosha, Jamuna & Prabhavathi, 2013; Shin, Choi, Chung, Kang & Sung, 2008a). Its medicinal benefits have also been extended to processed products such as dehydrated garlic, garlic oil macerate, aged garlic extract (Choi, Cha & Lee, 2014) and recently to black garlic (BG) which is an Asian product considered one of the fastest-growing health foods (Zhang, Lei, Liu, Gao Xu & Zhang, 2015; Kimura et al., 2017, Ryu, & Kang, 2017). BG is produced by heat treatment of the raw garlic bulbs under controlled conditions of high temperature (of about 60 to 90 °C) and high relative humidity (50-95%) for long periods of time (several weeks) without any additional treatment or additives (Zhang, Li, Lu, Liu & Qiao, 2016). Although the origin of BG remains questionable, it is known that this product has been consumed since ancient times in Japan, Thailand and Korea; in 2014, the latter reported an estimated market value of BG of nearly 94 million US dollars (Bae, Cho, Won, Lee & Park, 2014).

The success of BG is related to the heating process that helps to minimise the main unpleasant characteristics of raw garlic such as pungent taste and odour, resulting in a sweet and aromatic product with a syrupy or gelatine-like texture. Taking into consideration the sensory properties of BG, it has been sold in different forms such as a whole BG, puree, powder, extract or capsule. The popularity of BG is increasing in Europe and America as an ingredient in high-end cuisine (Kim, Jung, Kang, Chang, Hong & Suh, 2012a), the food industry, alcoholic beverages, candies, ice creams, jams (Kim, Son, Kim & Kim, 2008; Lee, Kim, Kang, Kim, Lee & Ryu, 2010; Shin et al., 2008a), sausages (Yoon, Shin & Kang, 2014),

vinegar (Sim, Hwang, Kang, Kim & Shin, 2014), tofu (Sim, Hwang, Kang, Kim & Shin, 2014), white pan bread (Wang, Lee & Lee, 2013), jelly (Kim & Rho, 2011), pork ham (Yang, Shin, Kang & Sung, 2011) and yogurt bases (Shin, Kim, Kang, Yang & Sung, 2010). Also, due to its high antioxidant capacity, ==BG has been incorporated in cosmetic industry== in shampoos, facial creams, body soap and skin protectors (Kim, Jung, Kang, Chang, Hong & Suh, 2012b).

Changes in the physicochemical properties of BG undergone during the aging process, mainly involve modification or interactions affecting carbohydrates, volatile sulfur compounds, free amino acids, polyphenols and other antioxidant compounds. These changes are the main reasons for the enhanced bioactivity of BG compared with fresh garlic (FG) (Kim et al., 2011b). The magnitude of these changes depends largely on the heat treatment conditions and the reactions developed as a consequence of them, including non-enzymatic browning reactions such as the Maillard reaction (MR), the chemical oxidation of phenols, as well as the thermal degradation of organosulfur compounds (Bae et al., 2014; Zhang et al., 2015). There are currently few studies that have been published on changes in the carbohydrate fraction and formation of Amadori and Heyns compounds during the elaboration of BG (Ríos-Ríos, Vázquez-Barrios, Gaytán-Martínez, Olano, Montilla, & Villamiel, 2018; Yuan, Sun, Chen & Wang, 2016 & 2018) and all of them highlighted the critical role of MR in BG production. This reaction occurs between free amino groups of amino acids, peptides or proteins and carbonyl groups of reducing sugars that are present in the garlic bulbs (Gamboa-Santos, Megías-Pérez, Soria, Olano, Montilla, & Villamiel, 2014; Yu, Zhang, & Zhang, 2018). In this work, the chemical and physicochemical changes taking

place during the obtainment of BG have been reviewed, paying special attention to non-enzymatic browning reactions as the principal pathways that lead to the production of BG.

2. Chemical and physicochemical modifications

FG contains approximately 65% water, 28% carbohydrates, 2.3% organosulfur compounds, 2% proteins (allinase, among others), 1.2% free amino acids and 1.5% fibre (; Kimura et al., 2017; Santhosha et al., 2013) additionally, other minor constituents are present in a wide range such as primary and secondary non-sulfur biomolecules (steroidal glycosides, essential oils, polyphenols and vitamins B1, B2, B6, C and E) (Bozin, Mimica-Dukic, Samojlik, Goran & Igic, 2008; Samarth, Samarth & Matsumoto, 2017).

This composition is modified substantially during the manufacture of BG which consists of a thermal process at high relative humidity. Currently, the method for the production of BG is not standardised; processing conditions vary widely according to local traditions and the particular characteristics required in the end product, Table 1. As a result, there are different times and conditions for its manufacturing (Kim, Kang & Gweon, 2013; Kimura et al., 2017; Shin et al., 2008a; Toledano-Medina, Pérez-Aparicio, Moreno-Rojas & Merinas-Amo, 2016). Temperatures ranging from 60 to 90 °C and relative humidity values between 50-95% during the aging period between 22-35 days have been reported (Choi et al., 2014; Kim et al., 2008; Zhang et al., 2016). In different studies about BG elaboration its chemical transformation has been attributed to microbiological spontaneous fermentations as well as to non-enzymatic reactions. According to Sato, Kohno, Hamano and Niwano (2006) BG is produced after 40 days at 60-70 °C and 85-95% relative humidity by spontaneous

106   fermentation; however this affirmation was discarded by Wang, Feng, Liu, Yan, Wang,

107   Sasaki and Lu (2010) who did not detect any *Lactobacillus* species growth during the

108   incubation of BG at 70 °C, arguing that at high temperatures bacterial growth to promote the

109   fermentation process is not possible. Recently, Qiu, Li, Lu, Zheng, Zhang and Qiao (2018)

110   explored the microbial community during BG processing, and high microorganism

111   community abundance and diversity was observed. Although the correlation analysis showed

112   that the presence of some genera such as *Thermus* and *Bacillus* had positive correlation with

113   the reducing sugar, total phenol and total acid contents of BG, the authors indicated that the

114   chemical reactions, especially MR, play a crucial role in the processing of BG, as previous

115   research has reported. Thus, other authors have described the BG process as 'aging periods'

116   from 30 to 90 days under heat treatment suggesting that the transformation of raw garlic into

117   BG is due to non-enzymatic browning reactions, MR, caramelisation and the oxidation of

118   phenol (Bae et al., 2014; Chen, Kao, Tseng, Chang & Hsu, 2014; Choi et al., 2014; Miao,

119   Chen, Zhou, Xu, Zhang, & Wang, 2014; Zhang et al., 2015).

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121   *2.1. Changes in organosulfur compounds*

122   Garlic is a major source of sulfur containing compounds. A metabolic pathway for the

123   synthesis of these compounds in FG was proposed by Molina-Calle, Sánchez de Medina,

124   Priego-Capote and Luque de Castro (2017) who indicated that the first step is the formation

125   of γ-glutamyl-S-2-carboxypropyl-L-cysteine by the condensation of L-cysteine and

126   glutathione with the subsequent addition of methylacrylate to the thiol group of the L-

127   cysteine moiety. γ-Glutamyl-S-2-carboxypropyl-L-cysteine is the precursor of the γ-

128　glutamyl-S-(alk(en)yl)-L-cysteine group. Therefore, this compound and γ-glutamyl-S-

129　(alk(en)yl)-L-cysteine sulfoxide families lead to the formation of sulfoxides by S-

130　oxygenation and deglutamylation, respectively. On the other hand, sulfoxides, particularly

131　alliin (S-allyl-L-cysteine sulfoxide)m are broken down by the enzyme allinase (Cardelle-

132　Cobas, Moreno, Corzo, Olano, & Villamiel, 2005) giving rise to intermediate sulfenic acids

133　that condense to yield thiosulfinates. In whole raw garlic, the enzyme alliinase is localised in

134　vacuoles, separated from its substrate alliin; therefore when garlic is damaged (i.e.

135　contaminated by microbes, mechanically disrupted, ground) and the vacuoles are disrupted,

136　alliinase is released and rapidly hydrolyses alliin to produce allicin which is responsible for

137　the unpleasant flavour and odour of the garlic cloves (Bae et al., 2014; Ovesná, Mitrová &

138　Kučera, 2015; Rana, Pal, Vaiphei, Sharma & Ola, 2011). However, when garlic is subjected

139　to heat treatment above 60 °C, alliinase is inactivated and allicin production decreases; in

140　parallel, alliin and allicin suffer thermal degradation (Chen, Xu, Wang, Zhou, Fan, & Huang,

141　2017; Lawson, Wang & Papadimitriou, 2001).

142　　　　During the production of BG by heating at 70-80 °C for 10 days alliin decreased by

143　80% (Zhang et al., 2015).. This is due to allicin formation during the first two days and the

144　thermolysis process (Table 2). Alliin is especially susceptible to degradation at a high

145　temperature because of its unstable sulfoxide bond. Chen et al. (2017) studied the thermal

146　degradation of alliin at temperatures between 60 and 89 °C over 60 h and identified by HPLC-

147　MS secondary organosulfur compounds, mainly including S-allyl-L-cysteine (SAC) and

148　other ethers, such as allyl alanine disulfide, allyl alanine trisulfide, allyl alanine tetrasulfide,

149　dialanine disulfide (cysteine), dialanine trisulfide and dialanine tetrasulfide. SAC, the main

150　organosulfur compound, is formed during the enzymatic hydrolysis of γ-glutamyl-S-

allylcysteine, catalysed by γ-glutamyl transpeptidase (γ-GTP, EC 2.3.2.2). Nevertheless, although γ-GTP activity is affected by heat, Chen et al. (2017) showed a new pathway for the production of SAC from the direct thermal treatment of alliin, even though γ-GTP was inactive (Xu, Miao, Chen, Zhang & Wang, 2015). According to this, Bae et al. (2014) quantified by HPLC the same amount (113 μg SAC/g dry matter (DM)) in BG produced at 55 and 70 ºC, while in FG it was 20 μg SAC/g DM (Table 2). Similarly, Hanum, Sinha, Guyer and Cash (1995) reported a SAC content of about 20–30 μg/g in raw garlic and it increased 6-fold after the aging process. Recently Kim et al. (2017) reported 73.5 μg/g of SAC in FG juice and this value increase up to 242 μg/g in BG juice. Also Lawson and Hunsaker (2018) reported SAC as the main alliin metabolite in BG. In commercial aged BG extract, 1500 μg/g DM has been determined (García-Villalón et al., 2016). Another possible explanation for the decrease in alliin content was the MR, since this compound has a similar structure to amino acids which, together with reducing carbohydrates, are initial substrates of the reaction (Zhang et al., 2015), although this theory has not been confirmed. However, despite its thermal instability and reactivity, alliin has been found in BG methanolic extracts at a concentration of 300 μg/g (Chen et al. 2014).

On the other hand, allicin, with high reactivity and low thermal stability, is decomposed into diallyl sulfide (DAS), diallyl disulfide (DADS), diallyl trisulfide (DATS), dithiins, ajoene and finally is transformed into S-allylmercapto-cysteine (SAMC) a stable, tasteless and odourless compound that is, approximately, 6-fold higher in BG than in raw garlic (Corzo-Martínez et al., 2007; Zhang et al., 2015). The SAMC formation in BG has also been related to the enzyme γ-GTP which presents an optimal temperature for activity at 40 °C (Bae et al., 2014); therefore, during BG production, a complete inhibition of the γ-GTP is

174 expected. Hence, based on those studies, the increase in SAMC content can be mainly

175 attributed to allicin conversion.

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177 *2.2.* <mark>*Changes in carbohydrates. Non-enzymatic browning*</mark>.

178 Carbohydrates are the major constituents in the bulb of raw garlic. The polysaccharide

179 fraction of FG mainly consists of fructan, non-reducing water-soluble saccharides, which are

180 used by garlic as a carbohydrate reserve for osmoregulation, adaptation to low temperature

181 photosynthesis and protection from freezing stress (Chow, 2002; Darbyshire & Henry, 1981;

182 Fujishima et al., 2005). According to Losso and Nakai (1997) fructan concentrations range

183 from 125 to 235 mg/g on a wet weight basis, making up 96% of total non-structural garlic

184 carbohydrates. More recently, Judprasong Tanjor, Puwastien and Sungpuag (2011) analysed

185 different types of garlic for potential sources of fructans, obtaining contents of inulin between

186 224 at 292 mg/g wet weight (w/w), fructooligosaccharides (FOS) (kestose, nystose and

187 fructosyl-nystose; 9 at 16 mg/g w/w) and minor amount of fructose, glucose and sucrose (1-

188 2; 1-2 and 10-11 mg/g w/w respectively). Fructans are composed by β-$(2{\rightarrow}1)$ linked fructosyl

189 units with a terminal glucosyl moiety (Fig. 1) and a degree of polymerisation as high as 38

190 or even 50 (Baumgartner, Dax, Praznik & Falk, 2000; Darbyshire & Henry, 1981; Losso &

191 Nakai, 1997). Over 20 years ago, these compounds were recognised for their ability to modify

192 host microbiota to the benefit of the host's health (Gibson et al., 2017).

193 However, it should be pointed out that the carbohydrate fraction is degraded during the

194 production of BG. In a recent study, Li et al. (2017) showed a decrease of close to 30% in

195 the content of polysaccharides during BG production; the monosaccharide constituents of the

196 FG polysaccharide fraction were mainly fructose, galactose and galacturonic acid, at a molar

197 ratio of 307:25:32, while at the end of the process the polysaccharide fraction of BG had a

198 minor molecular mass and mainly contained galactose and galacturonic acid at a molar ratio

199 of 63:20. This indicates that fructan was completely hydrolysed during the process and the

200 remaining polysaccharide in BG consists of galactan and pectin (Li et al., 2017). According

201 to this, fructans are hydrolysed into its monomers, glucose (Fig. 1a) and fructose (Fig. 1b).

202 When these monosaccharides are heated, they undergo caramelisation or MR. Lu, Li, Qiao,

203 Qiu and Liu (2018) studied the effect of thermal treatment on soluble polysaccharide

204 degradation during black garlic processing and observed that high temperature accelerated

205 the degradation of polysaccharides to oligosaccharides and monosaccharides.

206 Garlic has a high protein content (19-14% DM) (Gamboa-Santos, Soria, Corzo-

207 Martínez, Villamiel & Montilla, 2012; USDA, 2017), with lectins, a very heterogeneous

208 group of glycoproteins, being the most abundant (Corzo-Martínez et al., 2007) and containing

209 all the essential amino acids (Banerjee, Mukherjee & Maulik, 2003), with glutamic acid (286

210 mg/100 g), arginine (409 mg/100 g), aspartic acid (90 mg/100 g) and tyrosine (449 mg/100

211 g) being the most abundant (USDA, 2017). During BG aging, the content of the amino acids

212 tyrosine (78 mg/100 g), arginine (71-40 mg/100 g) and glutamic acid (100 mg/100 g)

213 decreases, and, on the contrary, that of phenylalanine (136-143 mg/100 g) and methionine

214 (71-73 mg/100g) increases (Choi et al., 2014). However, Liang, Wei, Lu, Kodani, Nakada,

215 Miyakawa and Tanokura (2015) reported an increase of L-alanine, L-valine, L-isoleucine, L-

216 tyrosine, and L-phenylalanine at the early step of thermal processing up to 5 days and a

217 decrease during the later BG thermal processing of all free amino acids. The initial increase

218 most likely resulted from the degradation of proteins or peptides, which may result from

219 enzymatic and non-enzymatic hydrolysis and the later decrease may be caused by MR.

220   During the production of BG Amadori (1-amino-1-deoxy-2-ketose) or Heyns (2-

221 amino-2-deoxy-1-aldose) rearrangement products are formed. These first stable products of

222 the MR are intermediate compounds in the formation of numerous advanced glycation end-

223 products, present in other products such as dehydrated and powdered garlic (Cardelle-Cobas

224 et al., 2005). Moreover, the colour changes of BG are due to the products of MR, usually

225 associated with the absorbance increases at 280 nm, 320–360 nm, and 420–450 nm,

226 corresponding to the initial, intermediate and final stages of MR respectively (Fig. 2). The

227 contents of Amadori and Heyns compounds in garlic have been measured either underived

228 by HPLC-MS/MS (Yuan et al., 2016 and 2018) or as the products of acid hydrolysis (*N*-ε-2-

229 furoylmethyl-amino acids (FMAAs) by ion-pair RP-HPLC-UV in dehydrated garlic

230 (Cardelle-Cobas et al., 2005) and BG (Ríos-Ríos et al., 2018). The total contents of the three

231 main Amadori (Fru-Pro, Fru-Val, and Fru-Leu) and Heyns (Glu-Pro, Glu-Val, and Glu-Leu),

232 compounds in five samples of Chinese commercial BG ranged from 762.5 to 280.6 μg/g of

233 product (Yuan et al., 2016). These authors determined the same compound during the thermal

234 processing of garlic incubated at 55 °C with 80% relative humidity for 90 days, reaching the

235 maximal formation after 70 days (around 260 and 40 mg/g of Amadori and Heyns

236 compounds respectively; beyond 70 days Amadori compounds are severely degraded.

237   Regarding 2-FMAAs, furosine (2-furoylmethyl-lysine), 2-furoylmethyl-arginine (2-

238 FM-Arg) and 2-furoylmethyl γ-aminobutyric acid (2-FM-GABA) were quantified in

239 commercial samples of BG with contents between 63 and 145 mg 2-FMAAs /100 g protein,

240 with furosine being the most abundant in all samples. In the same study a progressive increase

in the furosine as well as in 2-FM-Arg (2-furoylmethyl-arginine) content was observed during the 10 days of aging of pre-treated samples at 70 °C and 94% of relative humidity reaching values of around 110 mg 2-FMAAs /100 g protein. It was reported that the combination of temperature/time during the production of BG has a noticeable effect on the evolution of FMAAs, indicating that high temperatures and long drying times could lead to high values of FMAAs in BG. Despite the fact that both studies (Cardelle-Cobas et al., 2005; Ríos-Ríos et al., 2018) established the usefulness of FMAAs as quality indicators for the early detection of the MR, their data are not fully comparable because of dissimilarities in the analytical methodologies, manufacturing conditions and garlic varieties selected.

On the other hand, 5-hydroxymethyl furfural (5-HMF) and other furfural derivatives such as 5-(hydroxymethyl)-2-furoic acid (5-HMFA) are also formed during the manufacturing process of BG through the degradation of Amadori and Heyns compounds, during the intermediate stages of the MR. The content of these compounds can increase throughout the aging process of BG (Liang et al., 2015). Although it has been recently proposed that these compounds are harmful, the beneficial effects of BG, including antioxidant, anti-inflamatory, cytoprotective and antitumour effects have become increasingly apparent (Kim et al., 2011a; Li et al., 2015b). The formation of 5-HMF is correlated with the intensity of the black colour. When its content reaches about 4 g/kg, the colour of the garlic become black. Also, the maximum amount of 5-HMF produced at the end of the manufacturing process was estimated at about 5 g/kg, (Zhang et al., 2016). The final content of this compound was conditioned by the process, thus at higher relative humidity and temperature, higher HMF formation reaching 8 g/kg after 12 days at 75 ºC and relative humidity 85% (Sun & Wang, 2018).

264   5-HMF and 5-HMFA formed in BG can be derived from caramelisation or MR (Liang

265   et al., 2015), being the latter the most important reaction taking place, according to the

266   reaction conditions. HMF formation by caramelisation occurs without amine participation

267   during the processing of foods with high sugar content (e.g., jams and certain fruit juices), or

268   in wine production, although high temperature was not used (Pereira, Albuquerque, Ferreira,

269   Cacho, & Marques, 2011). As BG is rich in fructose and glucose, HMF could be ascribed to

270   sugar decomposition during the thermal treatments.

271   Finally, the formation of brown colour compounds is usually associated with the

272   advanced stage of the MR. Usually, a high temperature leads to an increase in the

273   consumption of reducing sugars and free amino acids groups, giving rise to the fast

274   development of the MR (Corzo-Martínez, Corzo, Villamiel & del Castillo, 2012). In

275   agreement, Zhang et al. (2015) reported these facts during BG aging. During heating in the

276   temperature range of about 60-90 °C and at 80-90% relative humidity for more than 30 days,

277   raw garlic underwent intensive non-enzymatic browning and caramelisation, and brown

278   tones appeared during the first days of heating; a few days later the colour changed to black

279   (Zhang et al., 2016). During thermal processing, while the content of fructans decreased

280   gradually, the content of reducing sugars (glucose and fructose) and sucrose increased

281   accordingly first and then remained constant or decreased at the end of the process due to

282   their participation in the formation of coloured compounds (Choi et al. 2014; Zhang et al.,

283   2015). Even though, during caramelisation different compounds of low molecular weight and

284   others with double conjugated linkages (Sengar & Sharma, 2014) that produce odours and

285   more coloured compounds are synthesised. Little attention has been paid so far to the

286   contribution of caramelisation to the non-enzymatic browning reactions of BG. Taking into

account the severe thermal conditions applied to FG, more research is needed in order to know the contribution of caramelisation to the loss and formation of different compounds that are present in BG.

2.3. Changes in phenols

The qualitative and quantitative content of polyphenols in garlic is important since some of its potential health-promoting effects are attributed to its high phenolic level; however, the phenolic composition of garlic varies greatly with genetic, agronomic and environmental factors (Waterer & Schmitz 1994; Beato, Orgaz, Mansilla & Montaño, 2011). Moreover, Xu, Ye, Chen and Liu (2007) observed that heat treatments could lead to an increase in the free phenol forms from phenolic compounds, ester, glycoside or ester-bound fractions or from other supramolecular structures containing phenolic groups. In addition, the type of phenolic compounds present in the product can be affected or not depending on their thermosensitivity and the heating conditions of the process. This would in part explain the large variation in the reported polyphenols content of BG (Beato et al., 2011) another factor could be the different methods and units used for their measurement (Table 3).

Regarding changes in total phenolic content (TPC) during the manufacture of BG all studies agree that TPC significantly increases, so that BG contains a level of polyphenols several-fold higher than FG (Kim et al., 2013; Toledano-Medina et al., 2016; Kimura et al., 2017). Usually TPC is determined spectrophotometrically by the Folin-Cicalteu method and expressed as gallic acid equivalents (GAE). TPC in BG has been ranged between 4-9 mg/g (Kim et al., 2013; Kim et al., 2017), 12-15 mg/g (Zhang et al., 2016), 15-24 mg/g (Li et al.,

2015a) and 25-58 mg/g (Choi et al., 2014). In the study developed by Kim et al. (2013), TPC increases 9-fold more in the last step of BG production and hydroxycinnamic acid derivatives (HCA) increased 4-fold more at different processing steps (Table 3), with respect to FG. In another study, Toledano-Medina et al. (2016) reported an increase from 5,130 mg GAE/kg DM to 14,850 mg GAE/kg DM; this may be related to the higher antioxidant activity of BG (ABTS radical method, 110 mmol Trolox/kg lyophilised sample) than FG (16 mmol Trolox/kg lyophilised sample). Values of the same order of magnitude were reported by Zhang et al. (2016) over 60-90 ºC during 69-9 days and by Li et al. (2015a) after a freezing pre-treatment during BG production (Table 3).

Also, similarly to polyphenols, the total flavonoid content (TFC) can vary as a consequence of processing conditions. Using colorimetric methods, values around 2-fold higher were detected in BG as compared to FG (Table 3) (Choi et al., 2014; Kim et al., 2013; Kim et al., 2017). Among the major flavonoid subgroups in FG, flavones and flavanols were found at the highest concentrations, and for BG, they were flavanols and flavonols (Kim et al., 2013).

Furthermore, Sasaki, Lu, Machiya, Tanahashi and Hamada (2007) and Choi et al. (2014) reported that raw garlic bulbs present aromatic amino acids such as tyrosine (449-340 mg/100g) that decreased dramatically during the processing of BG (77-170 mg/100 g) which could be ascribed to the synthesis of phenolic acids and the high amount of them in BG.

3. Sensory properties

During the manufacturing of BG process important organoleptic changes take place; the garlic turns black and acquire a soft texture and a sweet taste, even though the non-existence of a single method for the production of BG has resulted in a product of variable quality (Bae et al., 2014). Furthermore, the absence of a quality index means that it is necessary for each producer to decide which characteristic is the best for BG including the colour, taste, texture and content of bioactive compounds (Fig. 3).

Few data have been reported on organoleptic properties concerning moisture content. Ríos-Ríos et al. (2018) compared the loss of weight in garlic samples pretreated at 68 °C during 6 days and 56 °C for 12 days using an air tray dryer; the results showed that around 40% of the initial garlic weight was lost as water. It can be inferred that those temperatures did not cause a considerable damage in the garlic cell wall since the tissue could be rehydrated when samples were subjected to 70° C/ 94% of relative humidity and the production of BG was achieved after 10 days. On the other hand, the use of drastic conditions to shorten times, such as freezing (-18 °C) and high temperatures (90 °C), probably causes more considerable degradation of the cell wall polysaccharides (Liang et al., 2015; Li et al., 2015a) resulting in softer tissue in the BG and, therefore, in variable gum-like textures.

In spite of the lack of texture parameters, generally, it was reported that moisture values between 40-50% are recommended for a softer product; when the moisture content is around 40%, it seems that BG could be much drier and its elasticity will be poor. In particular, when the moisture content goes below 35%, BG becomes too hard to eat (Zhang et al., 2016). In general, the rate of moisture removal increases with temperature and the pH also decreases more significantly in garlic heated at high temperatures (Bae et al., 2014). Besides moisture changes, a continuous decrease in pH from initial levels of around 6.3-6.2 to values of 4.0-

353   3.7, depending on the processing conditions, was produced (Kang, 2016; Zhang et al., 2016),

354   with this decrease being accompanied by an increase in total acids content (5 g/kg in FG and

355   31-38 g/kg in BG); this fact may be related to the bittersweet taste in BG. Sun and Wang

356   (2018) studied the evolution of acids present in BG elaborated at 65, 75 and 85 °C and

357   observed that the highest acid concentration (6%) was obtained at 75 °C after 12 days.

358   Although one of the purposes of the study on the BG process is to shorten the aging

359   time using temperatures close to 90 °C, the organoleptic properties of the end-product must

360   be taken into account. Thus, comparing the effects of heat treatments at 60, 70, 80 and 90 °C

361   and 80% relative humidity, above 80 °C BG does not have an appropriate sweet flavour

362   because the consumption of excessive amounts of reducing sugar results in the development

363   of undesirable off-flavours (Zhang et al., 2016; Kimura et al., 2017). On the other hand,

364   heating at 60 °C in BG does not develop a suitable and homogeneous black colour and total

365   acid content does not increase, so the BG does not acquire a good sour flavour. The sensory

366   score in terms of colour, flavour, texture, taste quality and general acceptability is

367   significantly higher in BG aged at 70 °C (Zhang et al., 2016). In agreement, Lee, Yang and

368   Ryu (2011) reported at 70°C a sensory evaluation comprising colour, flavour, appearance

369   and overall acceptability of BG and results showed that the longer aging time, the better

370   evaluation. Moreover, BG from a process that includes a freeze pre-treatment and then

371   heating processing at 70 °C, showed for the 21st–24th day the highest sensory evaluation by

372   10 panel judges, indicating that for a good quality BG product the process should be stopped

373   on the 22nd day (Li et al., 2015a). Also, an increasing rate of browning related to the formation

374   of 5-HMF (Li et al., 2015a) as well as an important increase in the concentrations of water-

375   soluble sugars from FG at 188% to BG at 791% were obtained (Yuan et al., 2016). Hence,

the sweetness of BG has been associated with the increase in the content of fructose due to the complete hydrolysis of fructans. It was reported that sucrose and glucose have no contribution in the sweetness of BG since their content remains almost equivalent in FG and BG, probably due to the fact that fructans are composed of many molecules of fructose and only one of glucose and because glucose is an aldose that reacts more quickly with amino acids in the MR than ketoses do (Yuan et al., 2018).

Another important change produced during BG production is related to the pungency typical characteristic of raw garlic and is attributed to allicin which is responsible for the unpleasant flavour and odour in the garlic cloves (Bae, Cho, Won, Lee & Park, 2012; Ovesná et al., 2015; Rana et al., 2011). However, as it was mentioned in section 2.1, when garlic is subjected to heat treatment above 60 °C alliinase is inactivated and allicin production decreases between 75 and 95% diminishing the pungency compounds in BG (Lawson et al., 2001; Zhang et al., 2016; Chen et al., 2017). The concentration of derivative compounds from S-alk-(en)yl-L-cysteine decreased, except allyl methyl sulfide, while flavour compounds increased (Fig. 3), especially those related with a roasted flavour (furfural, 3-methylbutanal, 2-methylbutanal), sweet flavour and spiciness; while the green and floral flavour 2-butenal was totally degraded and benzeneacetaldehyde was formed (Kim et al., 2011c; Molina-Calle et al., 2017). These facts provide evidence of the important role of processing conditions on the sensory properties of BG.

Conclusions remarks and future trends

Despite the fact that BG is known since ancient times in Asia, there is an emerging interest all around the world on its consumption due to the singular organoleptic

characteristics and bioactive properties. The questionable origin of BG and the non-existence of a single method for its elaboration have given rise to the absence of quality index that makes it difficult to achieve a standardised BG product. The thermal processing of BG results in physicochemical changes that mainly affect carbohydrates, sulfur amino acids and polyphenols compounds. Carbohydrate fraction is degraded during the production of BG and reducing sugars can participate in non-enzymatic browning. Allinase is inactivated by heat and alliin and allicin concentration decreases. Regarding changes in polyphenols, during BG manufacture, total phenolic content increases, so that BG contains higher level of polyphenols than the fresh product.

This review is focused on the chemical and physicochemical modifications paying special attention to the non-enzymatic browning reaction as one of the main changes to obtain BG. These reactions play a crucial role in the processing of BG and determine the typical organoleptic characteristics of this product; in a similar way other authors have described the BG process as 'aging periods' under heat treatment. Furthermore, current researchs on the formation of Amadori and Heyns compounds have demonstrated that the transformation of BG is mainly due to non-enzymatic browning reactions, MR, instead of spontaneous generation produced by microorganisms. Findings presented in this work show the usefulness of MR compounds such as 2-furoylmethyl-amino acids and HMF as indicators of quality for controlling the non-enzymatic process of BG as well as evidence of the important role of the conditions of moisture and temperature required for the industrial production of BG. However, organoleptic properties of the final product must be studied in more depth in future works with controlled conditions in order to establish a clear relation between chemical and sensory characteristics of BG.

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661

662 **Figure captions:**

663 **Fig. 1.** Proposed mechanism from raw garlic fructan to black garlic by non-enzymatic

664 reactions: a) Amadori b) Heyns pathways (Adapted from Baumgartner, Dax, Praznik & Falk,

665 2000), with permission from Elsevier.

666

667 **Fig. 2.** Relation between the content of Amadori and Heyns compounds and the elaboration

668 time of black garlic. (Adapted from Yuan, Sun, Chen & Wang, 2018), with permission from

669 Elsevier.

670

671 **Fig. 3**. Major compounds present in black garlic.

672

673