

## Review

# A critical review of current technologies used to reduce ginkgotosin, ginkgotosin-5'-glucoside, ginkgolic acid, allergic glycoprotein, and cyanide in *Ginkgo biloba* L. seed

Isaac Duah Boateng

Division of Food, Nutrition and Exercise Sciences, University of Missouri, 1406 E Rollins Street, Columbia, MO 65211, United States

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## ABSTRACT

The *Ginkgo biloba* has astonished scholars globally with enormous bioactives, with sales exceeding \$10 billion since 2017. The *Ginkgo biloba* seed (GBS) is an essential part of culinary culture. Nevertheless, toxins in fresh *Ginkgo biloba* seed (GBS) have limited GBSs' daily consumption. Ginkgotosin and ginkgotosin-5-glucoside cause poisoning, tonic-clonic convulsions, and neurotoxic effects. Ginkgolic acid causes cytotoxicity and allergies. Allergic glycoprotein in GBS causes nausea, seizures, dyspnea, mydriasis, vomiting, and bellyache. The amygdalin-derived hydrocyanic acid cause dizziness, vomiting, cramping, and sleeping disorders. Food products are frequently exposed to various processing techniques to increase food safety and functionality. As a result, this review focused on the technologies that have been used to minimize toxins in GBS. In addition, a comparison of these techniques was made based on their benefits, drawbacks, feasibility, pharmacological activities, and future direction or opportunities to improve current ones were provided.

## 1. Introduction

The *Ginkgo biloba* L. (GB) tree (Fig. 1A) is a remarkable relict species and the only survivor of the Ginkgoaceae family within the Ginkgophyta plant lineage, whose ancestors may be traced back 250 million years (Boateng & Yang, 2020; Liu, Lu, Gao, Li, & Yang, 2022; Wang & Zhang, 2019). The GB tree is native to China, Japan, and Korea, but it has also been grown in Europe, the United States, and temperate regions of New Zealand, India, and Argentina (Boateng & Yang, 2022; Nabavi & Silva, 2019; Omidkhoda, Razavi, & Hosseinzadeh, 2019). The name 'ginkgo' is a linguistic translation of the tree's Japanese name, while the 'biloba' refers to the tree's two distinct leaf lobes (Boateng & Yang, 2022). The phylogenetic position of GB among the Spermatophyta has long been debated (Liu et al., 2022). The GB, on the other hand, is unique among living Spermatophyta in that it has kept some plesiomorphic reproductive traits, which explains why the species was given the qualifier "living fossil" (Leistner & Drewke, 2010; Liu, Wang, Zhang, & Wang, 2021; Liu et al., 2022). The GBs is very similar to that of its

extinct ancestors, as it has a plethora of compounds such as bilobalide, ginkgolides, biflavonoids, ginkgolic acids, carotenoids, triterpenes, essential oils, polyphenols, D-glucaric acid, glycerol, ascorbic acid, aromatic acids, lipids, alkyl coumarins, long-chain hydrocarbons, and carbohydrates, with distinct structures due to its distant phylogenetic relationship with other plants (Boateng, Soetanto, Li, Yang, & Li, 2021; Boateng & Yang, 2022, 2021d; Leistner & Drewke, 2010; Liu et al., 2022). The chemical diversity of GB has increased as a result of these substances.

The GB is a broadly used medicinal plant because of its abundant bioactives with wide-range pharmacological effects. Therefore, nearly all parts of GB (Fig. 1A–E) have been subjected to extensive research. The GB extract (GBE) is among the most sold and studied medicinal herbs, as it contains phenolic compounds, ginkgolides, and flavonoids. These components help with cardioprotection, blood circulation, antioxidant, anti-cancer, neuroprotection, and anti-inflammation effects (Boateng & Yang, 2022; Liu et al., 2021; Shen et al., 2021). The GB is widely utilized in functional foods, pharmaceutical, acupuncture, and

**Abbreviations:** GB, *Ginkgo biloba* L.; GBE, *Ginkgo biloba* L. extract; GBL, *Ginkgo biloba* L. leaves; MPN, Ginkgotosin; MPNG, Ginkgotosin 5'-glucoside; GA, Ginkgolic acid; GBSP, *Ginkgo biloba* L. seed protein; PM, Pyridoxamine; PL, Pyridoxal; PN, Pyridoxine; PMP, Pyridoxamine-5'-phosphate; PNP, Pyridoxine-5'-phosphate; PLP, Pyridoxal-5'-phosphate; GAD, glutamate decarboxylase; GABA,  $\gamma$ -aminobutyric acid; CFU, Colony forming unit; HPLC, High-performance liquid-chromatography; IC<sub>50</sub>, half maximal inhibition concentration; LD<sub>50</sub>, Median lethal dose; SSF, Solid-state fermentation; SmF, Submerged fermentation; MST, Membrane separation technology; MW, Molecular weight; FAO, Food and Agriculture Organization; WHO, World Health Organisation.

E-mail addresses: [boatengisaacduah@gmail.com](mailto:boatengisaacduah@gmail.com), [idbhpy@missouri.edu](mailto:idbhpy@missouri.edu).

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cosmetics industries worldwide, with sales exceeding \$10 billion since 2017 (Liu et al., 2022). In 2020, sales of GB extract (GBE) for North America were more than \$1 billion (Dziwenka and Coppock, 2021).

Compared to GB leaves (GBL), the GB seeds (GBS) (Fig. 1E) have a long history of use (Herbals first documented them in 1350 CE) and are used as food and medicine (Boateng & Yang, 2022; Wang & Zhang, 2019). The GBS is a vital component of Asian food culture in nations like Korea, Japan, and China and is also used in fruit candies, beverages, and others (Boateng, Soetanto, Yang, et al., 2021; Shen, Shi, Wu, Fan, & Li, 2020). In 2020, GB's export turnover in China was \$1,812,754, according to China's General Administration of Customs (Liu, Zou, Wang, Cao, & Su, 2021). By removing the hard shells and thin endopleuras (Fig. 1C), the edible parts of GBS are soft yellow seeds (Fig. 1E) that can be used to treat cough, asthma, neurological dysfunctions, and intestinal tract worm infections (has medicinal usage in Chinese Pharmacopoeia). Besides, GBS is applied in traditional Chinese medicine for treating kidney and lung problems (Boateng & Yang, 2022; Li & Hu, 2015).

Despite this, toxic chemicals in fresh GBS have limited their use in everyday life (Boateng, Yang, & Li, 2020; Scott, Lau, Lawrence, & Lewis, 2000). Ginkgotoxin (Fig. S1), also referred to as *n*-methyl pyridoxine (Scott et al., 2000; Yoshimura et al., 2006), is a vitamin B6 analog that causes poisoning, tonic-clonic convulsions, vomiting, and neurotoxic consequences by lowering the brain's  $\gamma$ -aminobutyric acid (GABA) (Boateng & Yang, 2022; Leistner & Drewke, 2010; Salamon, Gurgui, Leistner, & Drewke, 2009; Wang & Zhang, 2019). Ginkgotoxin (MPN) concentrations in fresh GBS range from 170 – 404  $\mu\text{g/g}$ , and medical toxicology reveals that MPN concentrations of more than 170  $\mu\text{g/g}$  cause toxicity (Barceloux, 2008; Boateng & Yang, 2020, 2021a). Ginkgotoxin 5'-glucoside (Fig. S1), identified by Scott et al. (2000), is also toxic. Kobayashi, Yoshimura, Johnno, Sasaki, & Wada (2011) stated that ginkgotoxin 5'-glucoside's (MPNG) toxicity in mice was only a quarter of ginkgotoxins' (MPN) toxicity, and this was corroborated in 2019 by Kobayashi (2019).

Ginkgolic acid (Fig. S2) is cytotoxic (Liu & Zeng, 2009; Ryckewaert, Sacconnay, Carrupt, Nurisso, & Simões-Pires, 2014), mutagenic (Berg, Braun, Krug, & Schrenk, 2015), genotoxic (Berg et al., 2015; Ryckewaert et al., 2014), allergenic (Chen et al., 2020; Hausen, 1998; Kochibe,

1997), and neurotoxic (Ahlemeyer, Selke, Schaper, Klumpp, & Krieglstein, 2001; Yang, Wang, Li, & Ma, 2014). As a result, the 2020 edition of the European, United States, and Chinese Pharmacopoeia state that the maximum amount of ginkgolic acid (GA) in GB extract is 5  $\mu\text{g/g}$  (Chen, 2021; Chinese Pharmacopoeia Commission., 2020; European Pharmacopoeia Commission., 2020; United States Pharmacopoeia Commission., 2020). Furthermore, the allergenic glycoprotein in GBS causes allergic reactions in humans (Yang & Wu, 2009; Zhou, Wang, Ye, Tao, et al., 2016). As a result, clinical symptoms such as diarrhea, convulsions, mydriasis, nausea, vomiting, dysphoria, bellyache, dyspnea, and even mortality in children may occur, restricting the development of GBS protein (Sun & Yang, 2019; Yang et al., 2011; Zhou, Wang, Ye, Chen, et al., 2016). Moreover, GBS has lethal hydrocyanic acid generated from amygdalin, and consuming high amounts of GBS can cause dizziness, vomiting, cramping, and sleeping disorders (Boateng & Yang, 2020, 2022; Boateng, Yang, & Li, 2020; Lim & Kim, 2018b). After swallowing GBS, a female (6-year-old) was admitted to the hospital and treated for cyanide poisoning, as convulsions and metabolic acidosis were witnessed (Cho, Kim, & Kim, 2003; Shin et al., 2020).

Food products are frequently exposed to various processing techniques (enzymatic hydrolysis, thermal, non-thermal, hybrid processing, etc.) to increase food safety and functionality (Costa, Mafra, Carrapatoso, & Oliveira, 2012; Costa et al., 2021; Knorr & Augustin, 2021; Mendes, Costa, Vicente, Oliveira, & Mafra, 2019). These processing methods such as thermal treatments (frying, boiling, roasting, and blanching), pressure (high-pressure processing and high-pressure micro fluidization), irradiation (pulsed ultraviolet, high-voltage impulses, gamma-radiation, and pulsed electric fields) causes protein aggregation, unfolding, and other chemical alteration (Costa, Mafra, Carrapatoso, & Oliveira, 2016; Costa, Oliveira, & Mafra, 2013; Costa et al., 2021; Zhou, Wang, Ye, Chen, et al., 2016), that might reduce or increase food allergenicity by destroying or hiding allergenic epitopes (depending on the nature of protein) (Costa et al., 2021), and reducing or increasing the toxic components (Boateng & Yang, 2021a; Boateng, Yang, Tahany, Li, & Yolandani, 2020; Guo et al., 2018; Koszucka, Nowak, Nowak, & Motyl, 2019; Zou, Zhang, Wu, et al., 2021). Because GBS can be eaten, a literature review on the various technologies used to reduce

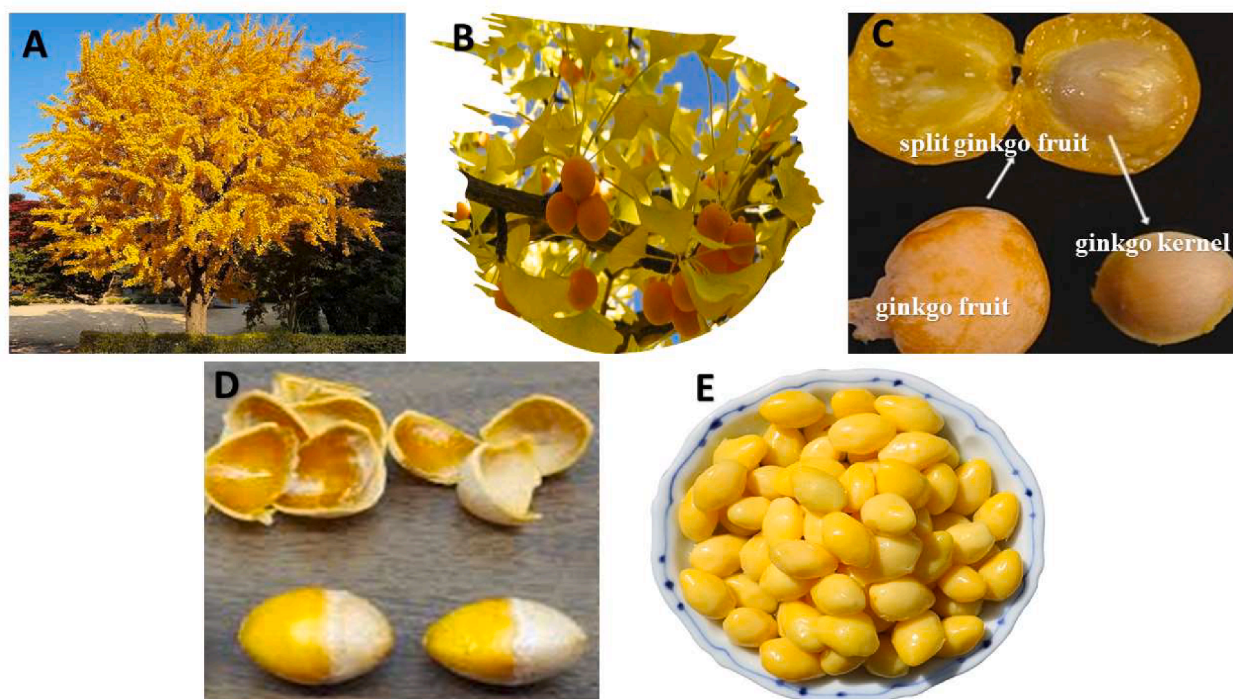


**Fig. 1.** The GB tree and GBS's various parts The ginkgo A. tree; B. fruit; C. fruit showing the split fruit and kernel D. kernels and seeds E. GB seeds.

ginkgotoxin, ginkgotoxin-5-glucoside, ginkgolic acid, allergenic glycoprotein, and cyanide in GBS is essential.

Hence, this review focused on the technologies that have been used in the previous decade to minimize toxins and allergens (ginkgotoxin, ginkgotoxin-5-glucoside, ginkgolic acid, allergenic glycoprotein, and cyanide) in GBS. Comparisons of these techniques were made based on their benefits, drawbacks, feasibility, and future trends. In addition, the effect of these technologies on the GBS's pharmacological activities were presented. This article will help food scientists, food engineers, technicians, physicians, pharmacists, chemists, and academicians understand the function of these technologies in decreasing toxic components, their drawbacks, and future improvements.

## 2. Ginkgotoxin (MPN)

### 2.1. Overview

In 1881, two Japanese boys died from ginkgo seed poisoning, according to the first case record (Kudo, 1981). The GBS poisoning was characterized by loss of consciousness, clonic or tonic convulsions, and vomiting. After World War II, when there was a scarcity of food, the first peak of the occurrence happened in the 1940 s and 1950 s (Leistner & Drewke, 2010; Mei et al., 2017; Salamon et al., 2009). However, the components responsible for the intoxication could not be identified until Wada, Ishigaki, Ueda, Sakata, & Haga (1985) isolated ginkgotoxin from GBS for the first time in 1985, confirming that it is the primary toxin responsible for acute poisoning symptoms. Wada et al. (1985) found that guinea pigs exhibited convulsions 30–40 min after receiving 6 mg/kg of MPN injections and that an intraperitoneal injection of 30–50 mg/kg of MPN might cause ventricular fibrillation, atrioventricular block, and death. Ginkgotoxin was also found in *ginkgo biloba* leaves (GBL) (Leistner & Drewke, 2010; Salamon et al., 2009), contrary to an earlier study by Wada et al. (1985) that it was only identified in GBS. The GBL extract (EGb761) is used to produce GB medicines. As a result, the toxin can be found in GB medicines, homeopathic preparations, and processed GBS (Boateng & Yang, 2022; Leistner & Drewke, 2010; Wang & Zhang, 2019).

Vitamin B6 and MPN are derivatives because of similar structural properties (Kobayashi, 2019; Kobayashi et al., 2015, 2011; Leistner & Drewke, 2010; Zhu et al., 2021). The analogs of vitamin B6 (Fig. S1) are mainly six interconvertible vitamins (water-soluble) which are dependent on pyridoxine (PN) (Salamon et al., 2009), pyridoxamine (PM) (Leistner & Drewke, 2010), and pyridoxal (PL) (Sado et al., 2019) besides their 5'-phosphorylated types (pyridoxamine-5'-phosphate (PMP), pyridoxal-5'-phosphate (PLP), and pyridoxine-5'-phosphate (PNP)) (Boateng & Yang, 2022; Gong et al., 2019; Leistner & Drewke, 2010; Zhang, Shi, Zhang, Cao, & Su, 2021). The PM, PL, and PN are initially metabolized to terminal metabolites (4-pyridoxic acid, PMP, and PLP) (Boateng & Yang, 2022; Zhang et al., 2021; Zhu et al., 2021), and they are essential coenzymes for over 230 enzymes that are involved in the metabolism of amino acids, carbohydrates neurotransmitters, and lipids metabolism, and have a crucial role in the regulation of antioxidant activity, anti-tumor, and immune responses (Boateng & Yang, 2021a, 2022; Boateng, Yang, & Li, 2020; Kobayashi, 2019; Kobayashi et al., 2015, 2011; Leistner & Drewke, 2010; Sado et al., 2019).

Nonetheless, the vitamin B6 derivative (MPN) induces GBS's poisoning by inhibiting glutamate decarboxylase (GAD), an enzyme in the brain which synthesizes  $\gamma$ -aminobutyric acid (GABA) using pyridoxal phosphate as a cofactor; consequently, MPN reduces GABA (Gong et al., 2019; Kobayashi et al., 2011; Leistner & Drewke, 2010). Because MPN is consumed by ingesting GBS and has a high propensity for human pyridoxal kinase, the GAD activity was inhibited when PLP levels were low (Sado et al., 2019). The ginkgo seed's poisoning has been documented in Korea, China, and Japan, presumably since the ginkgo seed has long been ingested as a traditional cuisine by Japanese, Koreans, and Chinese (Boateng, Soetanto, Yang, et al., 2021; Boateng & Yang, 2021b,

2022; Mei et al., 2017). The GBS poisoning is characterized by vomiting, tonic-clonic seizures, and coma (Di Lorenzo et al., 2015; Boateng et al., 2020; Mei et al., 2017).

Numerous research has found adverse effects, including the presence of MPN in patients who have been exposed to GBS's poisoning (Di Lorenzo et al., 2015; Jang, Roh, Jeong, Kim, & Sunwoo, 2015; Kobayashi, 2019; Kobayashi et al., 2015; Leistner & Drewke, 2010; Mei et al., 2017; Sado et al., 2019; Yoshimura et al., 2006). In some patients, several studies have demonstrated unfavorable consequences. A 2-year-old girl developed sudden recurrent tonic-clonic seizures after swallowing 20–30 GBS, necessitating medical treatment (Sado et al., 2019). Vitamin B6 was measured in serum and cerebrospinal fluid, and high MPN was reported in both cerebrospinal fluid and serum. A Japanese woman (36 years) experienced numerous vomiting and severe convulsions, 4 h after eating 70–80 GBS (microwaved). Per the high-performance liquid-chromatography (HPLC) studies, the serum's MPN ranged from 240 to 1,280 ng/ml (Mei et al., 2017). After eating 50–60 roasted GBS, a Japanese girl (2-year-old) had vomiting, diarrhea, and irritability within 7 h, and her serum's MPN was 360 ng/ml after afebrile convulsion (Leistner & Drewke, 2010; Mei et al., 2017). Within 12 h of admission, a Japanese boy (2-year-old) had vomiting and afebrile seizures, and his blood's MPN was 37–157 ng/ml, with 397 ng/ml in his urine 18 h later. After consuming an unspecified number of GBS, a Swiss boy (23-month-old) suffered two afebrile tonic-clonic convulsions that were verified by measuring the MPN in his urine and blood (Di Lorenzo et al., 2015). A Korean woman (51-year-old) ate a considerable number of GBS (1 kg) in less than an hour (Boateng & Yang, 2022; Jang et al., 2015; Mei et al., 2017), and due to a low blood vitamin B6 level of 2.2  $\mu$ g/l (normal range is 5–50  $\mu$ g/l), she suffered a tonic-clonic convulsion and postictal disorientation after 12 h.

### 2.2. Current technologies used to reduce ginkgotoxin in GBS.

Pharmaceutical processing has been applied to minimize toxicity or adverse effects, improve purity, intensify beneficial effects, alter pharmacological activities, maintain active ingredients, enhance flavor and remove bad taste (Hong, Jang, Hwang, & Cho, 2017; Lim & Kim, 2018a). For the use of various technologies to reduce ginkgotoxin, thermal processing (frying, steaming, microwaving, boiling, drying, etc.), fermentation, adsorption technology, and hybrid technologies have been applied (Table 1).

In a study by Hong et al. (2017) on changes in MPN in GBS in different cooking conditions (boiling, steaming, and pan-frying), they found that the MPN decreased as the cooking time increased. In the case of boiling, the MPN started to decline significantly from 5, 15, and 35 min, but no significant difference in MPN at 35 and 45 min occurred. In the case of steaming, at 10 min, there was no significant difference compared to the sample before steaming, and the MPN started to decrease significantly from 30 min. Hong et al. (2017) reported that the MPN declined as the cooking time increased, but there was no significant difference after 30 min. There was no significant difference from 30 min until 90 min. Pan-frying was the most efficient method for reducing MPN, as the MPN significantly reduced after 8 min. This shows that the cooking methods and duration affect MPN reduction. This was consistent with Kobayashi et al. (2011) report, which found that MPN was much lower in microwave-heated and boiled GBS than fresh GBS, showing that the heating process had a role in the MPN variations. The potency of thermal processing in reducing MPN was also reported by Gong et al. (2020) when they studied the impact of various thermal processing (microwaving, boiling, frying, and baking) on MPN (Table 1). From their findings, all the thermal treatments decreased MPN. Frying and baking reduced MPN by 46.54–54.67% and 36.38–54.56%, correspondingly. As a result, frying was selected as the best thermal processing technology for lowering MPN. This aligns with the comparative studies by Hong et al. (2017), who also reported that pan-frying was the most efficient for reducing MPN. According to Gong

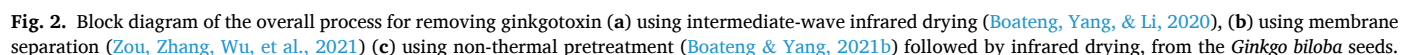
**Table 1**  
Technologies used to reduce ginkgotoxin (MPN) in ginkgo seeds.

| Technology        | Method                                                         | Conditions                                                                                                                                                                                                                                                                                                                           | MPN                                                                                                                                                                                                                                                       | Reference                            |
|-------------------|----------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------|
| Thermal           | Boiling                                                        | Time (min)<br>10, 15 and 20                                                                                                                                                                                                                                                                                                          | Reduced from 175.98 µg/g to 29.29, 24.71, and 32.16 µg/g                                                                                                                                                                                                  | Gong et al. (2020)                   |
|                   | Microwaving                                                    | Microwave power (Watts) and time (min)<br>100 W for 13 min<br>500 W for 5 min<br>1000 W for 3 min                                                                                                                                                                                                                                    | Reduced from 175.98 µg/g to 30.19 µg/g<br>30.60 µg/g<br>30.52 µg/g                                                                                                                                                                                        | Gong et al. (2020)                   |
|                   | Baking                                                         | Temperature (°C) and Time (min)<br>120 °C for 60 mins<br>180 °C for 20 mins<br>240 °C for 10 mins                                                                                                                                                                                                                                    | Reduced from 175.98 µg/g to 17.48 µg/g<br>15.70 µg/g<br>15.70 µg/g                                                                                                                                                                                        | Gong et al. (2020)                   |
|                   | Frying                                                         | Temperature (°C) and Time (minutes)<br>150 °C for 20 min<br>175 °C for 12 min<br>200 °C for 10 min                                                                                                                                                                                                                                   | Reduced from 175.98 µg/g to 17.91 µg/g<br>17.91 µg/g<br>19.88 µg/g                                                                                                                                                                                        | Gong et al. (2020)                   |
|                   | Heating of GBS extract solution                                | Temperature (°C) and Time (minutes)<br>90 °C for 15, 30, 45, and 60 min<br><br>120 °C for 15, 30, 45, and 60 min<br>150 °C for 15, 30, 45, and 60 min                                                                                                                                                                                | Reduced from 371.56 µg/g to 173.23, 160.65, 157.75, and 127.67 µg/g, respectively<br>Reduced from 371.56 µg/g to 155.87, 148.79, 151.82, and 136.51 µg/g, respectively<br>Reduced from 371.56 µg/g to 136.83, 127.96, 81.45, and 76.84 µg/g, respectively | Lim & Kim (2018a)                    |
|                   | Pan-frying                                                     | Time (min)<br>8, 11, 14, 17, and 20 min                                                                                                                                                                                                                                                                                              | Reduced from 350 µg/g to 280, 280, 290, 265 and 250 µg/g, respectively                                                                                                                                                                                    | Hong et al. (2017)                   |
|                   | Steaming                                                       | Time (min)<br>10, 30, 50, 70, and 90 min                                                                                                                                                                                                                                                                                             | The MPN decreased significantly from 30 min. However, after 30 min, the decrease was not significant ( $p > 0.05$ ) until 90 min                                                                                                                          | Hong et al. (2017)                   |
|                   | Roasting                                                       | Time (min) and Temperature (°C)<br>150 °C for 15, 30, 45, and 60 min<br><br>180 °C for 15, 30, 45, and 60 min<br><br>210 °C for 15, 30, 45, and 60 min                                                                                                                                                                               | Reduced from 198.05 µg/g to 188.31, 185.96, 185.04 and 177.86 µg/g, respectively<br>Reduced from 198.05 µg/g to 191.28, 188.06, 173.00 and 163.46 µg/g, respectively<br>Reduced from 198.05 to 192.06, 183.81, 155.85 and 52.88 µg/g, respectively.       | Lim & Kim (2018b)                    |
|                   | Drying                                                         | PVD (T = 80 °C VP/AP time = 10: 5)<br>HAD (T = 80 °C AV = 2 m/ s.)<br>IRD (T = 80 °C AV = 2 m/ s and H = 6 cm)<br>FD (Initially frozen (−20 °C, 24 h), and freeze-dried (T = −50 °C)                                                                                                                                                 | Reduced from 243.80 µg/g to 55.19 µg/g<br>53.19 µg/g<br>50.98 µg/g<br>53.15 µg/g                                                                                                                                                                          | Boateng & Yang (2020)                |
|                   | Optimizing IRD                                                 | T = 80 °C, t = 159.68 min, and H = 12 cm                                                                                                                                                                                                                                                                                             | Reduced from 243.8 µg/g to 50.57 µg/g                                                                                                                                                                                                                     | Boateng, Yang, & Li (2020)           |
| Fermentation      | Submerged fermentation using <i>Eurotium cristatum</i>         | inoculum size = $5.1 \times 10^5$ CFU/ mL pH = 5.8, medium volume = 100 mL and fermentation days = 4                                                                                                                                                                                                                                 | 40.15% of the ginkgotoxin was degraded                                                                                                                                                                                                                    | Zou, Zhang, Dong, et al. (2021)      |
|                   | Solid-state fermentation using <i>Eurotium cristatum</i>       | pH = 5.0, CFU/ g of inoculum size = $2 \times 10^8$ , sterilized water = 3 mL, fermentation days = 4                                                                                                                                                                                                                                 | Reduced from 260 µg/g to 250 µg/g                                                                                                                                                                                                                         | Zou, Cao, et al. (2021)              |
| Adsorption        | Resin adsorption                                               | Strong acid resins using<br>HD-8<br>HZ008<br>D001<br>732                                                                                                                                                                                                                                                                             | The MPNs' adsorption efficiency were<br>89.84%<br>88.14%<br>88.03%<br>87.59%                                                                                                                                                                              | Zhang et al. (2020)                  |
|                   | Membrane separation technology                                 | Extraction conditions<br>solvent = water cycle = 2, T = 25 °C, solid-liquid ratio 1:40 g/mL t = 20 min<br>Membrane filtration conditions<br>Ultrafiltration membrane with MW of with 10 kDa: 0.2–0.3 MPa with a permeate volume (4.7 L)<br>Nanofiltration membrane with MW of with 0.6 kDa: 0.4–0.5 MPa with a permeate volume = 3 L | All the ginkgotoxin could be removed                                                                                                                                                                                                                      | Zou, Zhang, Wu, et al. (2021)        |
| Hybrid technology | Combined endogenous enzymatic hydrolysis with resin adsorption | strong acid resin (D001) followed by endogenous enzyme(s); T = 40 °C, pH = 5.0 and t = 180 min.                                                                                                                                                                                                                                      | The MPN's adsorption efficiency reached $98.89 \pm 0.99\%$ .                                                                                                                                                                                              | Zhang et al. (2020)                  |
|                   | Drying followed by rehydration (60 °C in water)                | HAD-R, IRD-R, PVD-R and FD-R                                                                                                                                                                                                                                                                                                         | Reduced from 243.80 µg/g to 33.90, 32.82, 29.22, and 30.15 µg/g, respectively                                                                                                                                                                             | Boateng, Yang, Tahany, et al. (2020) |
|                   | Nonthermal pretreatment followed by IRD                        | Control (no pretreatment)<br>OS : sucrose (15% w/v), T = 30 °C, t = 30 min<br>OS + V: sucrose (15% w/v), 30C, VP/AP time = 10: 5 t = 30 min<br>US: PO:PF time = 2:10, t = 30 °C, PD = 100 W/l, F = 28 kHz and t = 30 min                                                                                                             | Reduced from 243.80 µg/g to 61.83 µg/g<br>41.67 µg/g<br>49.00 µg/g<br>41.33 µg/g<br>39.17 µg/g                                                                                                                                                            | Boateng & Yang (2021a)               |

(continued on next page)

| Technology | Method | Conditions                                                                                      | MPN | Reference |
|------------|--------|-------------------------------------------------------------------------------------------------|-----|-----------|
|            |        | OS + US: sucrose 15% w/v, PO:PF time = 2:10, t = 30 °C, PD = 100 W/l, F = 28 kHz and t = 30 min |     |           |

HAD, hot-air drying; PVD, pulsed-vacuum drying; FD, freeze-drying; IRD, infrared drying; FD-R, freeze-dried rehydration; IRD-R, infrared-dried rehydration; HAD-R, hot-air dried rehydration; PVD-R, pulse-vacuum dried rehydration; OS, osmotic; OS + V, osmovacuum; US, ultrasound; US + OSS, osmosonication pretreatment followed by infrared drying; VP/AP, vacuum and atmospheric pressure ratio; T, temperature; t, time; CFU, colony-forming units; PD, power density; PO: PF, the ratio of pulsed-on to pulsed-off time; MW, molecular weight.



et al. (2020), thermal impact, enzymatic actions, and leaching may produce changes in MPN, which can be influenced by heat treatments and technological factors. Thus, heating lowered the MPN because of the MPN's inactivation by the hydrolytic enzyme.

Roasting, a thermal treatment method, has also been used to reduce ginkgotoxicin (Lim & Kim, 2018b). The MPN in GBS depended on temperature and time in a study by Lim and Kim (2018) (Table 1). The MPN reduced considerably as the heat treatment time and temperature were increased compared to the fresh GBS ( $p < 0.05$ ). The MPN's decrease was inversely proportional to temperature. The MPN at 210 °C (52.88 µg/g) was about 70% lower than the fresh GBS (198.05 µg/g). The MPN reduced significantly ( $p < 0.05$ ) with rising temperatures (60 min at 150 °C, 45 min at 180 °C, and 30 min at 210 °C), implying that MPN reduced rapidly with increasing temperature. This could be due to the comparable chemical structure and metabolic processes of MPN and vitamin B6, as MPN is produced by the methylation of pyridoxine and pyridoxal-5-phosphate (Fiehe et al., 2000). As a result, the MPN's metabolic pathway might be similar to the other vitamin B6 decreased by heat. The results of Lim & Kim (2018b) complemented those of Kobayashi et al. (2011), who reported that MPN dropped significantly in boiled (100 °C, 5 min) and microwaved (45 s) GBS. As a result, the decrease in MPN by heat was identical to those reported by Lim & Kim (2018b).

Drying is a vital technology primarily applied to facilitate food transportation, prolong shelf-life, and this has been used to reduce ginkgotoxicin (Boateng & Yang, 2020, 2021d; Boateng, Yang, & Li, 2020; Boateng, Yang, Tahany, et al., 2020). As shown in Table 1, Boateng & Yang (2020) found that different drying methods reduced the ginkgotoxicin by fourfold. They observed that the pyridoxine (PN), used to counteract ginkgotoxicin (Sado et al., 2019), increased in the dried products compared to the fresh GBS. Besides, there was a higher PN for heat-treatment (infrared, hot-air, and pulsed-vacuum drying) than non-thermal (freeze-drying). They explained that the reason might be due to the thermal treatment that cleaved off glucoside bond of ginkgotoxicin-5-glucoside (Fig. S1) to ginkgotoxicin, which further demethylated the ginkgotoxicin to pyridoxine and other vitamin B6 derivatives (Fiehe et al., 2000; Leistner & Drewke, 2010; Lim & Kim, 2018a). This was in line with the findings of Lim & Kim (2018a), who studied pyridoxine and MPN in an MPN standard solution and a GBS extract solution after heat treatment to see if heat treatment reduced the toxic compounds in GBS. The MPN (in the standard solution) declined from 994.92 to 563.69 µg/g, and the MPN (in the GBS extract solution) decreased from 371.56 to 76.84 µg/g. Both reductions were higher with increasing time. Interestingly, the pyridoxine (PN) in the MPN standard solution and GBS extract solution increased with increased time and temperature in all groups (except for the 90 °C). Besides, the HPLC chromatogram confirmed the degradation of MPN into PN. Boateng, Yang, & Li (2020) further applied two-step multiple response optimization techniques to methodically optimize infrared drying settings for GBS (Fig. 2A) to achieve maximum total phenolic content and minimum MPN. Under these settings (Table 1), the MPN was reduced from 243.80 µg/g to 50.57 µg/g (5.45 fold), which was lower than their previous research (Boateng & Yang, 2020). Thus, Boateng, Yang, & Li (2020) suggested that these infrared dryer's settings could be applied to GBS processing. To elucidate the mechanism of MPN reduction by drying, Boateng, Yang, & Li (2020) applied response surface and perturbation analysis to visualize the relationship between independent factors (infrared drying parameters) and ginkgotoxicin (Fig. S3) by holding one factor at a zero level. From the response surface plot (Fig. S3), increasing temperature and time while reducing the infrared emitter's height (distance between the emitter and ginkgo seed) had a collective impact on MPN. Time, temperature, and infrared emitter's height cleaved the glucoside bond of MPNG and demethylated MPN to PN because of the infrared radiation's higher penetration. The result is in tandem with Lim & Kim, (2018a, 2018b) studies. Moreover, the perturbation plot by Boateng, Yang, & Li (2020) demonstrated a negative impact of increasing temperature and

time and decreasing infrared emitter's height on MPN. The curve's shapes for temperature and time were steeper than the infrared emitter's height; thus, MPN is more sensitive to time and temperature, which corroborates the above explanations.

Adsorption (using resin) and membrane separation (ultrafiltration and nanofiltration) technology has been applied for the reduction of ginkgotoxicin (Zhang et al., 2020; Zou, Zhang, Wu, et al., 2021). In a study by Zhang et al. (2020), they reported that resin adsorption technology (strong acid resins HD-8, HZ008, D732, and D001) could remove 87.59–89.84 % of MPN. Among the four resins, D001 had the highest adsorption efficiency. This could be explained that sulfonate groups ( $-\text{SO}_3\text{H}$ ) are circulated on the D001's surface, and the MPN is a pyridine alkaloid (Zhang et al., 2020). Thus, the pyridine ring's nitrogen atom can be merged with the sulfonate for easy MPN adsorption. Membrane separation technology (using ultrafiltration and nanofiltration), as shown in Fig. 2B, has been applied for MPN's removal by Zou, Zhang, Wu, et al. (2021). According to their findings, the extraction, ultrafiltration, and nanofiltration techniques (Table 1) could transfer MPN (99.88%) from the GBS powder. All of the ginkgotoxicin could be removed under these conditions. As a result, membrane separation technology (MST) may be a viable option for eliminating ginkgotoxicin.

Fermentation, a metabolic process using microorganisms, has been applied to reduce ginkgotoxicin (Zou, Cao, et al., 2021; Zou, Zhang, Dong, et al., 2021). The submerged fermentation (SmF) of GBS powder using *Eurotium cristatum* was investigated to see if it could be used as a processing technology (Zou, Zhang, Dong, et al., 2021). Under the optimum fermentation conditions (Table 1), 40.1% of ginkgotoxicin in GBS was reduced. The decrease in MPN was linked to the enzymatic effects of *Eurotium cristatum*, according to Zou, Zhang, Dong, et al. (2021). As a result, SmF using *Eurotium cristatum* can significantly improve GBS's functional value and safety. Nonetheless, further research needs to be done to understand how *Eurotium cristatum* caused the reduction in ginkgotoxicin. Solid-state fermentation has also been applied to ginkgotoxicin (Zou, Cao, et al., 2021). However, it must be noted that the reduction in ginkgotoxicin was not significant ( $p > 0.05$ ). Besides, comparing the decrease in ginkgotoxicin during thermal processing to fermentation, it was observed that fermentation has an insignificant or minute reduction in ginkgotoxicin (Table 1). Thus, the fermentation process can be collaborated with other removal technology of ginkgotoxicin to guarantee the safety of GBS in the future.

Hybrid technology, which involves the amalgamation of various processes, is used as a viable method to improve efficiency, cut costs and increase food and medicinal products' quality and have recently gained increasing interest (Alolga et al., 2021; Boateng & Yang, 2021b; Boateng, Zhang, Li, Saalia, & Yang, 2021; Dzah, Duan, Zhang, & Ma, 2021). For reducing ginkgotoxicin in GBS, various works of literature have shown that hybrid techniques are more effective than a single process (Boateng & Yang, 2021a; Boateng, Yang, Tahany, et al., 2020; Zhang et al., 2020). For example, in research by Boateng, Yang, Tahany, et al. (2020), they applied two-step hybrid techniques (drying followed by rehydration), as shown in Table 1. In research by Boateng, Yang, Tahany, et al. (2020), they reported that the MPN in GBS using hybrid technologies was lower than the single technology (Fig. 2A) by 35.62 to 47.06%. They explained that during drying (1st stage), there was a degradation of MPN into PN and other vitamin B6's analogs, causing a reduction of MPN in the dried-GBS (from 243.80 µg/g to 55.19 µg/g in dried-GBS). Furthermore, during rehydration (2nd stage), most of the MPN that was left after drying dissolved in the water. Hence, a significant reduction in ginkgotoxicin (from 55.19 µg/g in the dried-GBS to 29.22 µg/g in rehydrated-GBS) was achieved. A hybrid drying approach (combined non-thermal pretreatment and infrared drying) was compared to a control (no pretreatment before infrared drying) in another study by Boateng & Yang (2021a), as shown in Fig. 2C. They reported that the MPN in hybrid technology was 36.65% lower than the single technology (no pretreatment before drying). This might be that during pretreatment (1st stage); some MPN (water-soluble) might have leached into the aqueous medium

in the course of the pretreatment process, and the drying (2nd stage) caused MPN's demethylation. Thus, the influence of pretreatment followed by drying (hybrid technology) had a synergistic impact as it increased ginkgtoxins' demethylation rate. In a study by Zhang et al. (2020) using resin adsorption combined with enzymatic hydrolysis (hybrid technology) to remove MPN (Table 1), they reported that the adsorption efficiency for MPN could reach 98.89% when hybrid technique was applied compared to a single technique (only resin adsorption) which removed 87.59 to 89.84 % of MPN. Thus, hybrid technology is more effective in removing toxic components than a single technology. In summary, the above literature reviews show that various technologies can reduce ginkgotoxin in GBS. Nevertheless, hybrid technology and MST are the most potent.

Two theories are present to explain the reduction in ginkgotoxin in processed GBS. According to (Lim & Kim, 2018b, 2018a), heat-induced demethylation converts ginkgotoxin to pyridoxine during the processing of GBS. The methylation of vitamin B6 phosphate derivatives, PN, and its analogs produce MPN in GBS (Fiehe et al., 2000; Leistner & Drewke, 2010). Lim & Kim (2018a) confirmed the decrease in MPN and an increase in PN was in consonance with the structural conversion of MPN to PN owing to its demethylation under various heat treatments. This trend was corroborated in Boateng & Yang (2020) and Boateng, Yang, & Li (2020) studies, during which there was an increase in PN and a significant decrease in ginkgotoxin. The other theory is by the enzymatic hydrolysis mechanism, as stated by Zhang et al. (2019) in which, endogenous  $\beta$ -glucosidase, which can catalyze the hydrolysis of ginkgotoxin-5-glucoside to ginkgotoxin in fresh GBS, is inactivated during processing. Hence, further research is required to understand the mechanism of these two theories in processed GBS and understand which one explains well in GBS processing.

### 3. Ginkgotoxin-5-glucoside (MPNG)

#### 3.1. Overview

Scott et al. (2000) discovered the 5'-glucoside derivative of ginkgotoxin (ginkgotoxin-5'-glucoside, MPNG) in the GBS in 2000. This was followed by Yoshimura et al. (2006), who synthesized the MPNG (Fig. S1) in GB. The MPNG has an antagonistic influence on vitamin B6's metabolism, similar to MPN (Kobayashi et al., 2011). The MPNG was given orally to mice in a study by Kobayashi et al. (2011), and the toxicity of MPNG was compared to MPN to see if MPNG is accountable for GBS's poisoning. The oral injection of MPN at 0.2 mmol/kg b.w. elicited convulsions in all mice (all 6 mice). Notwithstanding, the same dose of MPNG was ineffective in causing convulsions (no mice had convulsions). Seizures occurred in 100% of mice after oral administration of MPNG at 0.8 mmol/kg b.w., whereas five out of six mice died 2–3 h later. These findings suggested that MPNG had a fatal effect as well. Nonetheless, its toxicity was lesser than MPN. The mean onset times of convulsions for MPN (0.2 mmol/kg b.w.) and MPNG (0.8 mmol/kg b.w.) were 27 and 83 min, respectively. Furthermore, the time of death and the beginning of convulsions after MPNG (0.8 mmol/kg b.w.) injection were substantially longer than MPN (0.2 mmol/kg b.w.). These findings revealed that developing the toxic effect of MPNG took longer than developing the poisonous effect of MPN. Thus, ginkgotoxin is the major toxicant, not ginkgotoxin-5-glucoside (MPNG).

This echoes the medical toxicology statement that MPN's concentrations of  $>170 \mu\text{g/g}$  cause toxicity (Barceloux, 2008; Boateng & Yang, 2020, 2021a) and not MPNG. Besides, in a lot of case reports, concentrations of MPN and not MPNG are measured in the patients' serum and urine (Hasegawa, Oda, Ichiyama, Hori, & Furukawa, 2006; Kobayashi, 2019; Kosaki, Naito, Nojima, & Nakao, 2020; Sado et al., 2019). Nevertheless, the review on the current technologies used to reduce MPNG in processed GBS is of great importance.

#### 3.2. Current technologies used to reduce ginkgotoxin-5-glucoside in GBS

Fermentation (submerged and solid-state) has been applied by researchers to reduce MPNG in GBS (Table S1). However, in solid-state fermentation (SSF, microorganisms grow on solid materials without the presence of free liquid) by Zou, Cao, et al. (2021) using *Eurotium cristatum*, the reduction in MPNG was not statistically significant ( $p > 0.05$ ). Nonetheless, when submerged fermentation (microorganisms in the presence of plenty of free liquid medium) was applied using the same microorganism (*Eurotium cristatum*), the MPNG dropped by 40.15% than the fresh GBS. In the SmF, the conditions favorable for the *Eurotium cristatum* to degrade MPNG was higher than in SSF.

Adsorption technique has been applied to eliminate MPNG. In research by Zhang et al. (2020), various resins were applied (Table S1) to adsorb MPNG. The reduction in MPNG using adsorption technology was higher than the SmF stated above. Among the various resins, a significant difference in the adsorption efficiencies (AE) for MPNG was reported. For example, the AE of D001 was the highest (80.26%), while D732 (54.69) was the least. This shows that the kind of resin employed in adsorption technology affects the reduction rate of MPNG. To further improve the adsorption efficiency of D001, Zhang et al. (2020) applied a hybrid technology (Table S1) by combining endogenous enzymatic hydrolysis (EEH) with resin adsorption. Their results showed that the adsorption efficiency of MPNG reached 85.43% compared to only resin adsorption (80.26%). This could be explained that MPNG could be converted entirely to MPN under EEH, thus, resulting in a significant reduction. Membrane separation technology (using ultra and nano-filtration) was applied for MPNG removal by Zou, Zhang, Wu, et al. (2021), as shown in Fig. 2B. As shown in Table S1, all the MPNG could be removed. Thus, membrane separation is more potent than the other technologies for MPNG removal and holds great prospects for industrial application.

### 4. Ginkgolic acid (GA)

#### 4.1. Overview

Non-isoprenoid *n*-alkylphenols, known as ginkgolic acids (Fig. S2) that are salicylic acid derivatives, are present in the ginkgo seeds and leaves. The side-chains carbon atom number at the sixth position can be 13 to 17 (Fig. S2), and the side-chains double bond number can be 0 to 3, which is a homologous mixture (Fu, Hong, Li, & Liu, 2013; Wu et al., 2015; Yang et al., 2014). Therefore, the GAs include GA (13:0), GA (15:1), GA (15:0), GA (17:2), and GA (17:1) (Fig. 3A).

The neutral red assay was used to investigate the cytotoxicity of five ginkgo extracts with varying amounts of GA (0.35 to 58%) in human and animal cell lines (Siegers, 1999). The GA and the cytotoxicity of the extracts were highly correlated, with the extract with the lowest concentration of GA and having the highest  $\text{IC}_{50}$  (half-maximal inhibitory concentration) in treated cells, thus, showing low hazardous potential. However, the extracts'  $\text{IC}_{50}$  differed by 22–63 fold, with the lowest and highest GA. As a result, GA has been identified as highly hazardous.

As a result, the 2020 edition of the European, United States, and Chinese Pharmacopoeia stated that the maximum amount of GAs in GB extract is  $5 \mu\text{g/g}$  (Chinese Pharmacopoeia Commission., 2020; European Pharmacopoeia Commission., 2020; United States Pharmacopoeia Commission., 2020). The resazurin reduction study revealed that three primary GAs with a distinct alkenyl or alkyl groups (GA 13:0, 15:1, and 17:1) were lethal at concentrations  $\geq 50 \mu\text{M}$  in male Chinese hamster's lung fibroblasts (Berg et al., 2015). The GAs'  $\text{IC}_{50}$  was significantly lower than Egb761 in the HaCaT (human keratinocyte cell line) and the rhesus monkey kidney tubular epithelial cell line (LLC-MK2), measured by a neutral red uptake assay (4.6 vs. 1,481 mg/l in LLC-MK2 and 21.8 vs. 889 mg/l in HaCaT). Thus, indicating higher cytotoxicity (Hecker, Johannisson, Koch, & Siegers, 2002). The GA (1–100 mg/l) also enhanced the fraction of apoptotic cells in HaCaT cells and caused a

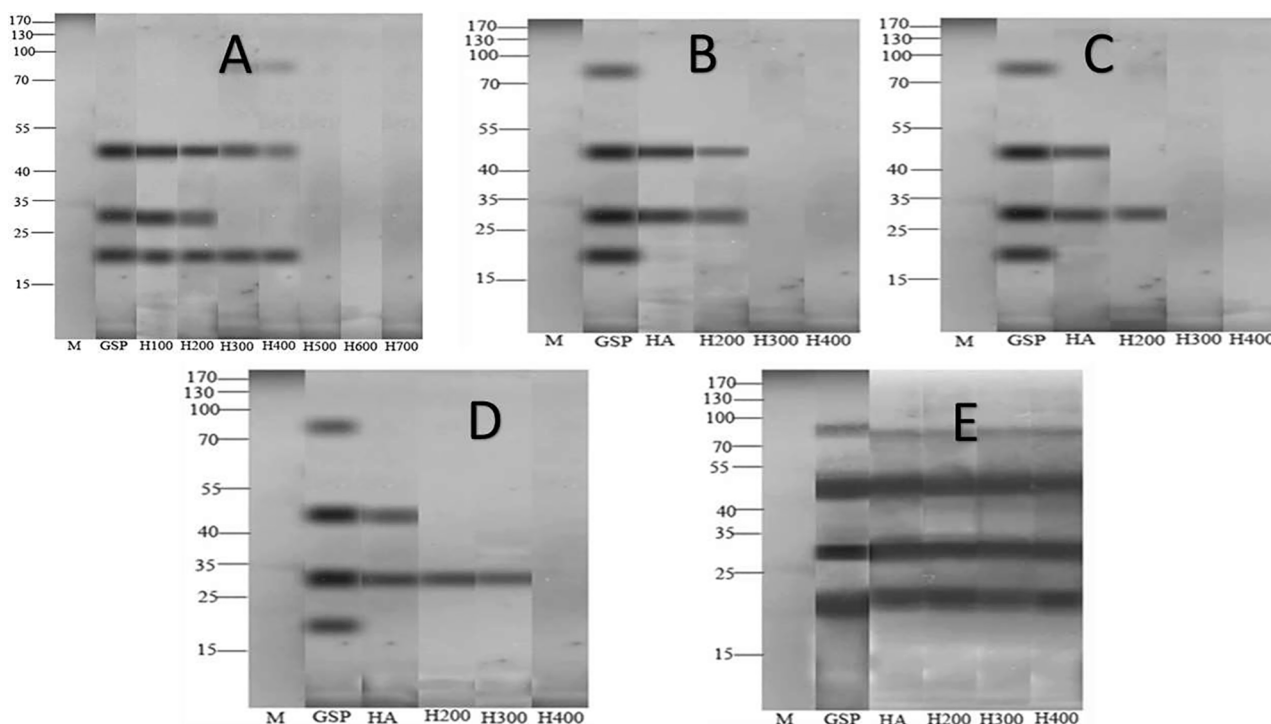


**Fig. 3.** Western blotting of native and HHP-treated GBSP (A). native GSP and its hydrolysates obtained by hydrolysis with papain (B), alcalase (C), pepsin (D), and neutrase (E). (Zhou, Wang, Ye, Chen, et al., 2016; Zhou, Wang, Ye, Tao, et al., 2016) **Note** M: Molecular weight standard solution; GSP: ginkgo seed proteins; high hydrostatic pressure (HHP) at H100: 100 MPa; H200: 200 MPa; H300: 300 MPa; H400: 400 MPa; H500: 500 MPa; H600: 600 MPa; H700: 700 MPa.

dose-dependent release of lactate dehydrogenase in LLC-MK2. Furthermore, the GA caused the production of myelinosomes in the cytoplasm of HaCaT cells, whereas LLC-MK2 cells transformed mitochondria due to uncoupled oxidative phosphorylation, according to morphological evaluations by electron microscopy. The GA also caused DNA strand breakage in primary rat hepatocytes and demonstrated neurotoxic effects in main cultures of neurons from chick embryo's telencephalons in the absence or presence of serum (Ahlemeyer et al., 2001). Chick neurons treated with GA (250  $\mu$ M) for 24 h showed apoptosis, like nucleus shrinkage and chromatin condensation. Protein phosphatase 2C $\alpha$  was found to be substantially activated in a follow-up mechanistic research, resulting in GA-induced neuronal death. The GA's embryotoxic impacts were validated by comparing the LD<sub>50</sub> (death of 50% of chick embryos) of three GB extracts' fractions containing various GA in fresh viable Leghorn eggs (Baron-Ruppert & Luepke, 2001). As a result, removing or lowering GA throughout the production process reduced the hazardous potential dramatically, necessitating a review of current technologies.

#### 4.2. Current technologies used to reduce ginkgolic acids in GBS

Various studies such as fermentation, conventional heating methods (steaming, roasting, microwaving), drying (traditional and advanced drying), and hybrid techniques showed that the GA's in GBS could be reduced efficiently using various technologies (Table 2).

Fermentation is the most common method to reduce GA in GBS, as shown in Table 1. In Guo et al. (2018) research using *Bacillus natto* (magnetically-immobilized) (Table 2), they reported that the GA reduced to 2.2  $\mu$ g/g, and that was 8.3-times lower than fresh GBS (18.67  $\mu$ g/g). They explained that GA reduction might be due to *Bacillus natto* changing the structure of GA. Furthermore, Zou, Zhang, Dong, et al. (2021) used *Eurotium cristatum* for submerged fermentation, as shown in Table 2. Their findings showed 100% degradation of GA in fermented GBS. They speculated that the decrease in GA was due to *Eurotium cristatum*'s enzymatic effects, supporting the previous research (Guo

et al., 2018) that fermentation reduces GA. Solid-state fermentation (SSF), which involves microorganisms grown on a solid surface or solid substrate that has a very low moisture content, as a means for bioconversion of biomass, has been applied as a detoxification technology by Zhou et al. (2014) to degrade the GA by using Fungi (*Candida tropicalis* and *Aspergillus oryzae*) as shown in Table 2. Zhou et al. (2014) found that after SSF, GA decreased from 14.8 mg/g to 1.5 mg/g, indicating degradation of >90% of GA. They explained that the drop in GA suggests that the fungus changed the structure of GA. The higher protease content in the substrate is primarily due to microflora expansion since the microbial growth converted substrates to a protein via secreted enzymes and some biological substances that might break down GA. This reduction in proteins or amino acids released by microbes that decreased GA could be due to GA binding. Guo et al. (2019) also applied SSF using *Bacillus natto* (Table 2) for GA reduction. From Guo et al. (2019) research, the HPLC profile of GA in GBS before and after fermentation showed that 81% of the GA were degraded (from 20.67  $\mu$ g/g in fresh ginkgo seeds to 3.87  $\mu$ g/g in fermented ginkgo seeds). They explained that proteins, enzymes, or amino acids secreted by *Bacillus natto* could have converted the GA, which attests to Zhou et al. (2014). Thus, SSF enhanced the safety of GBS. In a study by Zou, Cao, et al. (2021), they applied SSF using *Eurotium cristatum* (Table 2). The results showed GA in fermented GBS decreased by 44.97%. It must be noted that the type of microorganism and the SSF conditions affects the rate of GA degradation, as the recent study by Zou, Cao, et al. (2021) reported a lower degradation of GA (from 16.81  $\mu$ g/g to 9.28  $\mu$ g/g, 44.97% reduction) compared to 81% reduction (from 20.67  $\mu$ g/g in fresh GBS to 3.87  $\mu$ g/g in fermented GBS) by Guo et al. (2019) and 90% by Zhou et al. (2014).

Conventional and advanced heating methods (Table 2) have been applied in GA reduction. Fan et al. (2017) employed various steaming and microwave treatments and compared them to the fresh GBS (Table 2). The GA was shown to be significantly reduced after heating under various conditions, with a 54.5% drop for steaming (75 min, 115  $^{\circ}$ C) and a 27% reduction for microwaving (6 min, 1000 W). Besides, GA in GBS decreased when the temperature increased (from 100  $^{\circ}$ C to

**Table 2**

Technologies used to reduce ginkgolic acid in ginkgo seeds.

| Technology          | Method                                                   | Conditions                                                                                                                                                                                                                                                                                                 | Ginkgolic acid                                                                                                                                                                                                                                                       | Reference                       |
|---------------------|----------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------|
| <b>Fermentation</b> | Solid-state fermentation using <i>Eurotium cristatum</i> | pH = 5.0, CFU/ g of inoculum size = $2 \times 10^8$ , sterilized water = 3 mL, fermentation days = 4                                                                                                                                                                                                       | Decreased from 16.81 µg/g to 9.28 µg/g (44.97% reduction)                                                                                                                                                                                                            | Zou, Cao, et al. (2021)         |
|                     | Submerged fermentation using <i>Eurotium cristatum</i>   | pH 5.8, inoculum size = $5.1 \times 10^6$ CFU/ mL, volue of medium = 100 mL 100 and fermentation days = 4                                                                                                                                                                                                  | Reduced from 1.34 µg/mL to zero                                                                                                                                                                                                                                      | Zou, Zhang, Dong, et al. (2021) |
|                     | Fermentation with <i>Bacillus natto</i>                  | Stirring rate = 150 rpm, T = 36.4 °C, particle size = 60 mesh, pH = 6.5, t = 43 h, beads-solid ratio = 1 g/g and liquid-solid ratio = 16.4 mL/g                                                                                                                                                            | Decreased from 18.67 µg/g to 2.2 µg/g                                                                                                                                                                                                                                | Guo et al. (2018)               |
|                     | Solid-state fermentation                                 | Inoculation = $1 \times 10^7$ fungi/ 5 g residues, maltose and peptone = 2% w/v, pH = 7, moisture content = 40%, T = 25 °C, and time = 4 days.                                                                                                                                                             | Decreased from 14.8 µg/g to 1.5 µg/g                                                                                                                                                                                                                                 | Zhou et al. (2014)              |
|                     | Solid-state fermentation using <i>Bacillus natto</i>     | Initial water content = 73%, relative humidity = 80%, T = 38 °C, inoculation volume = 18%, and t = 38 hr                                                                                                                                                                                                   | Decreased from 20.67 µg/g to 3.87 µg/g                                                                                                                                                                                                                               | Guo et al. (2019)               |
|                     | Fermentation using different lactic acid bacteria        | Lactobacillus acidophilus GA 15: 1 at a time of 0,12, 24, and 48 h GA 17: 2 at a time of 0,12, 24, and 48 h GA 15: 0 at a time of 0,12, 24, and 48 h GA 17: 1 at a time of 0,12, 24, and 48 h <i>Lactobacillus casei</i> GA 15: 1 at a time of 0,12, 24, and 48 h GA 17: 2 at a time of 0,12, 24, and 48 h | 2.14, 0.70, 0.58 and 0.51, respectively 0.23, 0.10,0.07 and 0.06, respectively 0.07,0.01, 0.01 and 0.01, respectively 0.68, 0.17,0.15 and 0.13, respectively 2.14, 0.76, 0.70 and 0.51, respectively 0.23,0.20,0.16 and 0.05, respectively 0.07,0.01, 0.01,and 0.01, | Wang et al. (2019)              |

**Table 2 (continued)**

| Technology               | Method                         | Conditions                                                                                                                                                                                                                                                                           | Ginkgolic acid                                                                                                                                                                                                 | Reference                            |
|--------------------------|--------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------|
| <b>Thermal</b>           | Steaming                       | GA 15: 0 at a time of 0,12, 24, and 48 h GA 17: 1 at a time of 0,12, 24, and 48 h <i>Lactobacillus plantarum</i> GA 15: 1 at a time of 0,12, 24, and 48 h GA 17: 2 at a time of 0,12, 24, and 48 h GA 15: 0 at a time of 0,12, 24, and 48 h GA 17: 1 at a time of 0,12, 24, and 48 h | respectively 0.68,0.19,0.16 and 0.12, respectively 2.14, 0.84, 0.64 and 0.53, respectively 0.23,0.16,0.15 and 0.14, respectively 0.07,0.05,0.04 and 0.03, respectively 0.68,0.23, 0.16, and 0.13, respectively | Fan et al. (2017)                    |
|                          |                                | Steaming temperature 100, 105, 110, 115, and 120 °C                                                                                                                                                                                                                                  | Decreased from 118 µg/g to 83,79,70, 65, and 60 µg/g, respectively                                                                                                                                             |                                      |
|                          |                                | Steaming time 15, 30, 45, 60, 75, and 90 min                                                                                                                                                                                                                                         | Decreased from 118 µg/g to 93,74, 67, 54, 50, and 44 µg/g, respectively                                                                                                                                        |                                      |
|                          | Microwaving                    | Microwave power 350, 600, and 1000 W                                                                                                                                                                                                                                                 | Decreased from 118 µg/g to 92, 88, and 82 µg/g, respectively                                                                                                                                                   | Fan et al. (2017)                    |
|                          |                                | Microwave time 2, 4, 6, 8, and 10 min                                                                                                                                                                                                                                                | Decreased from 118 µg/g to 98, 96, 82, 81, 81 µg/g, respectively                                                                                                                                               |                                      |
|                          | Drying                         | HAD (T = 80 °C AV = 2 m/ s.) IRD (T = 80 °C AV = 2 m/ s and H = 6 cm) PVD (T = 80 °C VP/AP time = 10: 5) FD (Initially frozen (−20 °C, 24 h), and freeze-dried (T = −50 °C)                                                                                                          | Decreased from 16.44 µg/g to 2.33 µg/g 4.51 µg/g 2.64 µg/g 3.92 µg/g                                                                                                                                           | Boateng & Yang (2020)                |
| <b>Hybrid technology</b> | Optimizing IRD                 | T = 80 °C, t = 159.68 min, and H = 12 cm                                                                                                                                                                                                                                             | Reduced from 20.12 µg/g to 2.19 µg/g                                                                                                                                                                           | Boateng, Yang, & Li (2020)           |
|                          | Drying followed by rehydration | HAD-R, IRD-R, PVD-R and FD-R                                                                                                                                                                                                                                                         | Reduced from 16.44 µg/g to 1.56, 1.00, 0.99 and 2.2 µg/g, respectively                                                                                                                                         | Boateng, Yang, Tahany, et al. (2020) |
|                          | Pretreatment followed by IRD   | Control (no pretreatment) OS : sucrose 15% w/v, T = 30 °C, t = 30 min OS + V: sucrose 15% w/v, 30C, VP/                                                                                                                                                                              | Reduced from 20.12 µg/g to 3.04 µg/g 2.43 µg/g 2.38 µg/g 0.79 µg/g 0.90 µg/g                                                                                                                                   | Boateng & Yang (2021a)               |

(continued on next page)

Table 2 (continued)

| Technology | Method | Conditions                                                                                                                                                                                                                                        | Ginkgolic acid | Reference |
|------------|--------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------|-----------|
|            |        | AP time = 10:<br>5 t = 30 min<br>US: PO:PF<br>time = 2:10, t<br>= 30 °C, PD =<br>100 W/l, F =<br>28 kHz and t<br>= 30 min<br>OS + US:<br>sucrose 15%<br>w/v, PO:PF<br>time = 2:10, t<br>= 30 °C, PD =<br>100 W/l, F =<br>28 kHz and t<br>= 30 min |                |           |

**Note**

HAD, hot-air drying; PVD, pulsed-vacuum drying; FD, freeze-drying; IRD, infrared drying; FD-R, freeze-dried rehydration; IRD-R, infrared-dried rehydration; HAD-R, hot-air dried rehydration; PVD-R, pulse-vacuum dried rehydration; OS, osmotic; OS + V, osmovacuum; US, ultrasound; US + OSS, osmosonication pretreatment followed by infrared drying; VP: AP, vacuum and atmospheric pressure ratio; T, temperature; t, time; CFU, colony-forming units; PD, power density; PO: PF, the ratio of pulsed-on to pulsed-off time; MW, molecular weight; LAB, lactic acid bacteria; GA, ginkgolic acid.

120 °C). The minimum GA was obtained under 115 °C with no substantial difference between 115 °C and 120 °C during steaming. The GA was decreased when steaming time increased (from 15 min to 90 min), suggesting that GA could be unstable to thermal treatment. Regarding microwave treatments, Fan et al. (2017) reported that the higher the microwave power, the lower the GA. The GA was reduced by 27.4% when the microwave power was increased to 1000 W. The GA gradually decreased as microwave time was increased (from 2 to 10 min). There were no significant variations between different times (from 6 to 10) under the strength of 1000 W. This shows that the rate of GA degradation depends on various conditions applied to affect the structure of GA. These findings agree with Yang et al. (2014) research in which they applied thermogravimetry–differential thermal analysis to observe the GA's thermal decomposition. They posited that at 70 °C, there was a significant breakdown of GA at 243 and 310 nm under ultra-violet spectrum with prolonged time, implying that the carboxyl chromophores were eliminated in the thermally decomposed derivative. The GA's monomers were GA15:1, C13:0, and C17:1, as determined by HPLC. As a result, microwave and steaming may effectively lower GA, which could be useful in the industrial sector.

Penultimately, drying has been applied to decrease GA. In research by Boateng & Yang (2020) using different drying methods (Table 2), they observed that the GA reduced by 4–8 folds amongst various drying techniques, which was within the safe range (<5 µg/g). Thus, GA's reduction could be caused by structural changes after drying and supports Yang et al. (2014) earlier research. Boateng, Yang, & Li (2020) further optimized the infrared dryer's setting (Table 2) and observed that the GA further decreased in the GBS under the optimum conditions by 10.25% compared to the previous study (Boateng & Yang, 2020). This could be due to the higher penetration ability of infrared radiation under the optimum conditions that caused the rate of GA's structural changes, thus, improving the reduction.

Finally, hybrid technology has also been applied in GA's reduction (Table 2). Boateng & Yang (2021a) used hybrid technology, as shown in Table 2. The GA was lower for the hybrid (non-thermal pretreatment followed by infrared drying) than the single treatment (no pretreatment before infrared drying). This shows that hybrid technology is more effective in GA's reduction than a single technology. It must also be noted that the type of hybrid technology affects GA's reduction rate, as ultrasound pretreatments had a lower GA as opposed to osmotic and

osmovacuum (Table 2). It's possible that ultrasonic cavitation degraded GA's structure (by producing microspores inside), resulting in a substantial loss of alkylphenols. This caused more exposure of GA during drying, resulting in the reduction. This trend was also witnessed in another hybrid technology (drying followed by rehydration) by Boateng, Yang, Tahany, et al. (2020). In their research (Table 2), the GA reduced from 16.44 µg/g in fresh GBS to 2.33 µg/g in the dried GBS (1st stage) and an additional decrease (2nd stage) to 0.99 µg/g in the rehydrated GBS (57.51% lower than the dried GBS). The results show hybrid technologies cause a more significant decrease in GA.

## 5. Cyanide

### 5.1. Overview

The GBS contains toxic hydrocyanic acid produced from amygdalin (Boateng & Yang, 2022; Shin et al., 2020). Most experts thought that the symptoms linked with cyanogenetic glycosides were the cause of GBS poisoning in the earlier days (Cho et al., 2003; Kudo, 1981). However, since cyanogenetic glycosides are not the GBS's predominant toxicant, other chemicals are likely to induce poisoning, as there are few instances of cyanide poisoning (Boateng & Yang, 2022; Wang & Zhang, 2019). Ingesting high amounts of GBS, on the other hand, might produce disturbances in the central nervous system, resulting in dizziness, vomiting, cramps, sleep issues, and a clouding of consciousness (Boateng & Yang, 2020; Boateng, Yang, & Li, 2020; Cho et al., 2003; Lim & Kim, 2018b; Shin et al., 2020). For example, after eating a substantial amount of GB, a female patient (6-year old) in good health came to the hospital in a semi-comatose state with apparent cyanosis signs (Cho et al., 2003). Nevertheless, as stated above, current studies have shown that it is not the primary toxicant. Regardless, minute cyanide can have other effects. Hence, reviewing the processing techniques used to reduce the minute amount is of great importance.

### 5.2. Current technologies used to reduce cyanide in GBS.

Drying, roasting, and boiling, along with rehydration, are some of the technologies used to minimize cyanide in GBS (Boateng & Yang, 2020; Boateng, Yang, Tahany, et al., 2020; Lim & Kim, 2018b; Shin et al., 2020). It can be observed in Table S2 that most of the technologies applied in cyanide reduction are thermal treatment and hybrid technology. The ginkgo seed was roasted at different temperatures (150 °C, 180 °C, and 210 °C) for different timeframes (0, 15, 30, 45, and 60 min) in Lim & Kim (2018b) study. According to their findings (Table S2), the total cyanide reduced when the roasting temperature and time increased (from 1.49 to 0.70 µg/g). This demonstrates that heat treatment decrease cyanide. Lim & Kim (2018b) also claimed that cyanide's reduction rate was affected by time and temperature. The 210 °C treatment resulted in a considerable reduction of over 50% (1.74 to 0.78 µg/g) after 30 min. Boateng & Yang (2020) used a plethora of drying procedures on the GBS (pulsed-vacuum, infrared, hot-air, and freeze-drying). They observed a substantial reduction ( $p < 0.05$ ) in cyanide content to values (0.24 to 0.39 µg/g) that were within safety limits (5 µg/g) for various seeds and fruits, validating the findings of Lim & Kim (2018b) report that heat processing influences GBS's cyanide. The reduction could be due to the inactivation of  $\beta$ -glucosidase (Shin et al., 2020), which prevents amygdalin from being converted to cyanide.

Moreover, it should be noted that recent research has shown that the wet-heating method is more effective than dry-heating in reducing cyanide. Shin et al. (2020) roasted (10 min, 200 °C) and boiled (10 min, 100 °C) GBS and juxtaposed it to fresh GBS. From their results (Table S2), GBS's cyanide was reduced via thermal treatment, while boiling (7.79 µg/g) reduced it more than roasting (18.89 µg/g). Shin et al. (2020) explained that since cyanides are water-soluble, the cyanide compounds might have dissolved in water during the boiling, thus causing its higher reduction.

Finally, hybrid technology has also been used to reduce cyanide, which was more effective than the single technology discussed above. For example, in a study by Boateng, Yang, Tahany, et al. (2020), they applied two-pot hybrid technology: 1. dried by different methods (infrared, freeze, hot-air, and pulsed-vacuum drying) followed by 2. rehydration (dissolving the dried GBS in water medium for 60 min at 60 °C). From their findings (Table S3), the cyanide in fresh ginkgo seed was 1.407 µg/g, then decreased to 0.24 µg/g in the dried ginkgo seed (1st stage) and an additional reduction to 0.083 µg/g in rehydrated ginkgo seed (2nd stage), which were within safety limits (5 µg/g). This attests to Lim & Kim (2018b) and Boateng & Yang (2020) statement that GBS's processing reduced cyanide. This shows that the cyanide in GBS was reduced more by hybrid technology (drying-rehydration) than by single technology (drying only). Furthermore, in rehydration, the solubility of cyanide enables it to leach in the water medium (Shin et al., 2020), thus causing a further reduction in cyanide. Besides, Boateng, Yang, Tahany, et al. (2020) results showed that the type of hybrid technology also affects cyanide's reduction rate, as infrared drying combined with rehydration had the least cyanide. In contrast, the hot-air drying combined with rehydration had the highest cyanide; thus, different drying methods affect cyanide's dissolution rate during rehydration. Therefore, hybrid technologies cause a more significant decrease in toxic components.

## 6. Allergic glycoprotein in GBS

### 6.1. Overview

Ginkgo seed protein (GBSP) has a reasonably high protein (10–15%), an adequate amino acid (AA), and essential amino acids (EAAs) than the FAO/WHO's standard, making it a high-quality protein, according to research (Zhou, Chen, Wang, Ye, & Chen, 2012; Zhou, Wang, Ye, Chen, et al., 2016). The GBSP does not contain any restricted AA, and it is high in sulfur AA like cysteine and methionine, which are often lacking in plant-based proteins (W. Liu et al., 2021). The GBSP also has a significant amount of arginine, aspartic acid, and glutamic acid. In addition, its tryptophan concentration is higher than the recommended FAO/WHO's level (1 g/100 g) (W. Liu et al., 2021). Besides, GBS has a greater EAA concentration than lupin and oat and is comparable to corn, soy, wheat, and brown rice (Gorissen et al., 2018). As a result, GBSP outperforms most protein-rich plants nutritionally. The molecular weights (MW) of GBSP vary from 10 to 100 kDa, according to Yang et al. (2011), with high intensity of five primary protein bands (MW ~ 10, 21, 32, 38, and 48 kDa).

The GBSP contains numerous biological activities (anti-aging, anti-oxidation, anti-bacterial, and anti-tumor), making it a valuable product (Huang et al., 2010; Wang & Zhang, 2019; Zhou et al., 2012). For example, bioactive proteins isolated from GBSP have been shown to have a broad anti-bacterial range in several studies (Jiao, 2014; W. Liu et al., 2021; Sawano, Miyakawa, Yamazaki, Tanokura, & Hatano, 2007; Wang & Ng, 2000). In addition, numerous types of albumin with an MW of 29.25 kDa were discovered to be a glycoprotein in research on proteins with anti-aging and anti-oxidation properties (Huang et al. 2004).

Ginkbilobin1 and Ginkbilobin2 are antifungal proteins extracted from GBS and indexed in UniProt Knowledgebase (W. Liu et al., 2021). Jiao (2014) noted a substantial inhibition of GBSP to *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Salmonella*, *Saccharomyces cerevisiae*, *Aspergillus niger*, and *Rhizopus*. Wang & Ng (2000) extracted a protein (ginkbilobin) and found out that ginkbilobin had strong anti-bacterial capability against *Mycosphaerella arachidicola*, *Fusarium oxysporum*, *Coprinus comatus*, *Botrytis cinerea*, and *Rhizoctonia solani*. Besides, ginkbilobin showed moderate antibacterial activity against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Escherichia coli*. Additionally, an antifungal protein was extracted and named Gnk-2 (Wang & Ng, 2000), which inhibited some fungi (*Trichoderma reesei*,

*Candida albicans*, and *Fusarium oxysporum*) but did not demonstrate antibacterial activity against *Escherichia coli* (Sawano et al., 2007).

Albumin, a type of protein in GBS, prevents cellular oxidation and removes reactive oxygen radicals (Huang et al., 2010; Wang & Zhang, 2019; Zhou et al., 2012). The GBS's albumin was extracted from a novel antioxidant protein (G4b), and it had a significant antioxidant effect. The G4b's IC<sub>50</sub> on 1,1-diphenyl-2-picrylhydrazyl and OH<sup>•</sup> was 100.7 and 78.1 µg/mL, respectively, while DNA injury had 27 µg/mL and superoxide anions had 23 µg/mL (Huang et al., 2010). A new 18 kDa protein was purified from GBS, with no equivalent protein in their database. This protein has a high antioxidant ability against free radicals (Zhou et al., 2012). The linoleic acid emulsion assay demonstrated higher activity than α-tocopherol, a significant decrease in antioxidant activity, and a strong Cu<sup>2+</sup> and Fe<sup>2+</sup> chelating ability, boosting its biological functions.

Notwithstanding, ingesting GBSP can lead to allergic reactions in humans. Clinical symptoms such as dyspnea, diarrhea, vomiting, mydriasis, dysphoria, nausea, bellyache, convulsions, and even mortality might occur in infants and children who are particularly sensitive to GBSP (W. Liu et al., 2021; Yang et al., 2011; Zhou, Wang, Ye, Chen, et al., 2016). Masaomi & Chikafusa (1994) were the first to clone the 11S storage cDNA from the GBS and examined the relationship amongst amino sequence structures and their subunits. Because immunogenicity was found in 32 and 36 kDa storage proteins (Yang et al., 2011), it is assumed that those proteins from GBS will have immunogenicity and may cause an allergic reaction in in-vivo (Wu et al., 2016) used a guinea pig to assess the allergenicity of ginkgo protein in their study. According to the findings, immunoglobulin G and E levels in the sera were substantially higher than in the controls. The footpads swelled noticeably, and the passive cutaneous allergy test returned positive. JianTing, Caie, YingYing, ShaoQian, GongJian, and HongMei (2010) studied the mouse model of GBS protein's allergy and found that GBS group and the positive control group had high IgE and IgG levels. These findings imply that GBS has allergenic potential.

### 6.2. Current technologies used to reduce allergic glycoprotein in GBS

Research on reducing and eliminating ginkgo seed protein (GBSP) allergy has been conducted in the last decade, thus helping the production of hypoallergenic GBSP and making it suitable as a precious protein source (Dong, Wang, & Raghavan, 2020; Sun & Yang, 2019; Zhou, Wang, Ye, Chen, et al., 2016). Yang et al. (2011) suggested that GBS's protein might contain allergens, as they used Western blotting to isolate three potential allergens with MW of 21, 32, and 36 kDa. In addition, they found out that the GBS protein allergens were glycosylated.

High-pressure processing (HPP), a non-thermal technology (100–800 MPa), is commonly used to improve food quality and safety (Dong et al., 2020; Zhou, Wang, Ye, Chen, et al., 2016). Foods are subjected to homogeneous and concurrent forces in all directions. The HHP, on the other hand, causes irreversible changes in the appearance of food because of the compression of water and air (Dong et al., 2020). The structure of related proteins may be altered in such a high-pressure environment. Because of the aggregation and denaturation of treated protein, the HHP has been explored to lower GBSP immunoreactivity (Zhou, Wang, Ye, Chen, et al., 2016).

Researchers have looked into the impact of HHP on GBSP's allergenicity (Zhou, Wang, Ye, Chen, et al., 2016). The results of Western blotting with a serum pool from a mouse having an allergy are displayed in Fig. 3A. Allergenic proteins (MW of 22, 30, and 46 kDa) were found in the raw GBSP. The GBSP showed a reduction in total IgE-reactive bands after HHP (Zhou, Wang, Ye, Chen, et al., 2016). All immunoreactive bands (22, 30, and 46 kDa) remained when proteins were treated at 100 and 200 MPa. However, the bands (at 30 kDa) disappeared after HHP (300 and 400 MPa), but there was a development of immunoreactive bands (at 90 kDa). No immunoreactive bands were detected when

proteins were treated at 500–700 MPa. The IgE-binding activities in HHP-treated GBSP were examined using the enzyme-linked immunosorbent assay (ELISA) to characterize the immunochemical reactivity further. The Fig. S4A depicts the results. The HHP-treated protein's immunoreactivity was lower than raw proteins. At an increased pressure (100–700 MPa), the immunoreactivity decreased. Protein's treated at 100, 200, and 300 MPa, had antigenicity reductions of 5%, 10%, and 42%, respectively. Nonetheless, after HHP of up to 400 MPa, the protein had a slight increase in immunochemical reactivity. There was a significant drop in antigenicity for HHP > 400 MPa: antigenicity was reduced by 95% at 500 MPa, while allergenicity was lost entirely at 600 and 700 MPa. According to the findings, HHP can successfully lower GBSP antigenicity.

In their subsequent research, Zhou, Wang, Ye, Tao, et al. (2016) used a combination of HHP (200, 300, and 400 MPa independently) and enzymatic hydrolysis (alcalase, papain, neutrase, and pepsin) to test the allergenicity of GBSP. Western blotting results using a serum pool from a mouse allergic to GBSP are presented in Fig. 3B–E. Allergenic proteins with MW's of 22, 30, 46, and 90 kDa were found in the raw GBSP. The full IgE-reactive bands in GBSP were reduced when hydrolysis was coupled with HHP. At 22 and 90 kDa, the immunoreactive bands were removed by hydrolysis with alcalase or papain (Fig. 3B) and C at 0.1 MPa (atmospheric pressure). The 30 and 46 kDa remained in papain-hydrolyzed GBSP at 200 MPa, but only the 30 kDa was visible (alcalase-hydrolyzed GBSP). Nevertheless, all immunoreactive bands vanished when HHP was performed at 300 and 400 MPa. The Western blotting pattern revealed no intact allergenic proteins with alcalase or papain at these pressures. The primary immunoreactive bands (22, 30, and 46 kDa) remained in pepsin-hydrolyzed GBSP (at 0.1 MPa), whereas the 90-kDa band dissipated. After 400 MPa, all immunoreactive bands in the hydrolyzed GBSP disappeared. At 0.1 MPa (atmospheric pressure), most immunoreactive bands were intact in neutrase-hydrolyzed GBSP (Fig. 3E) and did not differ significantly from raw-GBSP. The hydrolysates retained immunoreactive bands (22, 30, 46, and 90 kDa) after HHP, with just a few immunoreactive bands becoming less apparent.

Zhou, Wang, Ye, Tao, et al. (2016) study examined hydrolysates' IgE-binding activities using ELISA to further determine the residual immunochemical reactivity in GBSP hydrolysates (Fig. S4B). When compared to raw protein, HHP minimized most hydrolysate's immunoreactivity. In comparison to hydrolysis at 0.1 MPa (atmospheric pressure), HHP considerably reduced the immunogenicity of hydrolysates. At 300 MPa, the binding ability of papain and alcalase hydrolysates to IgE was decreased, resulting in the highest antigenicity reduction of 96.8% for alcalase and 95.5 % for papain. However, HHP at 400 MPa increased the immunochemical reactivity of hydrolysates by a minuscule amount. The immunoreactivity of pepsin hydrolysates reduced as pressure was increased. In the hydrolysates formed at 400 MPa, the binding ability to IgE was decreased, resulting in a maximum antigenicity reduction (95.2 %) and a near-complete loss of allergenicity. These findings correspond to the Western blotting profiles (Fig. 3B–E). According to the results, enzymatic hydrolysis of GBSP by pepsin papain or alcalase minimized allergenicity. The enzyme's ability to break the antigenic determinants in protein allergens is related to its specificity, associated with breaking the antigenic determinants (Zhou, Wang, Ye, Tao, et al., 2016). Therefore, the HHP application to GBSP before enzymatic hydrolysis may result in more significant allergenicity reductions than atmospheric pressure hydrolysis. The HHP is said to cause protein denaturation, structural unfolding, and the dissociation of specific proteins into sub-units, allowing conformational antigenic epitopes to be exposed to proteases that are triggered in enzymatic-hydrolysis. As a result, these antigenic epitopes could be more accessible to enzymes; thus, they must be eliminated.

Finally, Sun & Yang (2019) reduced GBS proteins' immunogenicity using moist heat via glycosylation process. The glycosylation conditions for the experiment were as follows. The protein to glucose ratios were 1:1, 1:2, 1:3, and 1:4, and glycation temperatures were varied at 30, 50,

70, 90, and 120 °C for 120 min. From their studies, the immunogenicity of GBSP decreased from 0.08% at 30 °C to 60.4% at 90 °C, and no browning occurred. When the temperature reached 120 °C, the glycosylation of the complex decreased significantly ( $p < 0.05$ ). This may be due to the higher temperature that increased reaction speed, resulting in increased browning. At 120 °C, the immunogenicity of GBSP was the lowest. Per their studies, the optimum conditions (at which there was a 64% immunogenicity reduction) were; glucose (1:3), glycosylation temperature (90 °C), and reaction time (60 min). This shows that these processing methods could be utilized to minimize the allergenicity of GBS protein. Amongst the processing methods, hybrid technology (HHP combined with enzymatic hydrolysis) and thermal treatment are more feasible.

## 7. Advantages and disadvantages of the current technologies

The conversion of agricultural goods into food products necessitates processing. Processing is a reliable method of feeding the world's growing population. Food processing turns raw agrarian produce into edible, safe, healthful, nutritious food products and preserve foods (Knorr & Augustin, 2021). As a result, food processing is critical for feeding the world's growing population.

Fermentation of GBS has had neutral or positive effects on toxicants. It can be seen that submerged fermentation (SmF) has an advantage over solid-state fermentation (SSF) (Table 1, 2 and S1). As shown in Table 1, in SmF, 40.15% of the ginkgotoxin was degraded than SSF, which had no significant impact on ginkgotoxin. Besides, the GA reduced from 1.34 µg/ml to zero in SmF compared to SSF, which decreased from 16.81 µg/g to 9.28 µg/g. A similar comparison can be made in SSF using *Bacillus natto* and SmF using *Bacillus natto* to reduce ginkgolic acid (Table 2). Nevertheless, SmF requires expensive media and equipment, complex and costly downstream processes, and difficulty in waste disposal. Thus, although it can reduce toxicants, further research is needed on how contamination levels in SmF can be substantially reduced. Furthermore, sensory analysis is required to determine consumer acceptance of GBS using SmF. For the SSF process, there can be an improvement in removing toxicants in the GBS by combining the SSF with some emerging technologies such as ultrasound, pulse-electric field, or pulse-magnetic field technology to guarantee the safety of the GBS products.

Adsorption technology is a surface process that leads to the transfer of a molecule from a fluid bulk to a solid surface. Various researchers have used adsorption technology (Zhang et al., 2020; Zou, Zhang, Wu, et al., 2021) to remove toxicants in GBS (Fig. 2B), Table 1, 2 and S1. From the different adsorption techniques, it can be seen that the membrane separation technology (MST) method was more effective compared to 1. resin adsorption (RA) and 2. combined endogenous enzymatic hydrolysis with resin adsorption (EEHRA). In MST, all the MPNG and MPN could be removed compared to RA and EEHRA technology. Besides, freeze–thaw stability (estimated with the % of syneresis as an index), which represents GBS powder's ability to endure the undesirable physical alterations throughout freezing and thawing cycles, was similar to undetoxified GBS. This indicates that MST has better freeze–thaw stability. Nonetheless, the protein content in MST was lower than that of undetoxified GBS, which might reduce the inhibitory effect. In addition, further research needs to be done on removing the fouling effects of MST, which is the most crucial problem encountered during MST. This fouling, specifically if it is difficult to get rid of, can substantially impede the permeation rate through the membranes and make them essentially unsuitable for such application. Furthermore, membrane processes (MP) frequently do not scale up to accept massive stream sizes. The MP typically consists of several parallel membrane modules, which must be replicated repeatedly to scale to larger feed rates (Gao et al., 2011). Therefore, expensive cleaning and regeneration schemes may be necessary for MST. Thus, affecting the industrial application. Furthermore, improving the shelf-life of the GBS powder is of great economic significance. Hence MST can be combined with other

technologies.

Heat-treated foods have a longer shelf life and better quality. According to Lim & Kim (2018) research, various heat treatments increase the content of physiologically active compounds. Several studies have been conducted on applying thermal technology on GBS's toxicants' (Table 1-2, and Figs. 2, S1-2). Boiling has been used to reduce ginkgo-toxin, as shown by Hong et al. (2017). Nevertheless, the bioactives (TPC, TFC, and antioxidant activity) were reduced. Thus, although boiling is the easiest method of processing (it does not require special equipment and skill) and the toxicant was reduced, its bioactive components were lost. Besides, water-soluble pigments may be lost, like vitamin C (Boateng & Yang, 2021d; Boateng, Zhang, et al., 2021). To ameliorate these defects, steaming (a method in which the food is cooked by the heat generated by steam) has been applied to reduce toxicants and takes a slightly longer time than boiling. Research by Hong et al. (2017) found that steaming had a better reduction of ginkgotoxin and higher retention of antioxidants, as the antioxidant activity increased when steamed for 70 and 90 min. Fan et al. (2017) also reported that GA reduced from 118 µg/g to 44 µg/g when steamed under various conditions. Thus, comparing steaming to boiling, the GBS's nutritional ingredients can be improved, and the toxic components also can be eliminated during steaming. However, steaming of GBS is a slow process of cooking. In research by Hong et al. (2017), using boiling, steaming, and pan-frying, they found that frying was the most efficient for reducing MPN. A similar trend was reported by Gong et al. (2020) that frying was the best treatment in reducing ginkgotoxin compared to microwaving, boiling, and baking. Besides, a comparative study by Hong et al. (2017) showed that the fried GBS had the highest TPC, TFC, and antioxidants and took a shorter time (8 and 11 mins). Nevertheless, oils are expensive, and it is not an economical cooking method. Besides, repeated use of heated oils may produce harmful substances and reduce the smoking point, which might affect the consumers' health. Also, fried GBS is consumed immediately or shortly after preparation because its shelf life is short. For example, according to Boateng, Soetanto, Yang, et al. (2021), China's enormous ginkgo farming has outstripped market demand (could reach as high as 60,000 tons per annum). As a result, there is an urgent need to identify innovative ways to take advantage of GBS abundance and use irrespective of the season and overcome the scenario where GBS utilization is limited to the early stages of home-cooked dishes. Thus, there is a need for technology to reduce toxic components, improve product quality, be cost-efficient, and increase shelf-life.

Drying is a popular food processing technology that allows dried products to be accessed and utilized regardless of the season. Hence, drying technology was applied on GBS to reduce the toxicants and improve the product quality (Boateng & Yang, 2020; Boateng, Yang, Tahany, et al., 2020). As discussed in sections 2, 4, and 5, various drying methods could safely reduce toxic components. Nonetheless, different drying methods showed multiple impacts on various indexes. It was found that, for ginkgo seeds, the infrared drying method required the shortest time and had the highest energy efficiency (Boateng, Soetanto, Yang, et al., 2021). In addition, infrared dried ginkgo seed had the highest antioxidant activity, higher product quality, and bioactives (Boateng & Yang, 2020, 2021c). Therefore, infrared drying was the preferred method for drying ginkgo seed. The infrared drying conditions were optimized using response surface methodology (Boateng, Yang, & Li, 2020). Based on the findings, it was suggested that these infrared drying settings could be used for GBS processing. It must also be mentioned that infrared had some adverse effects on product quality (texture, vitamin C, soluble solids, and free amino acids). Hence, advanced technologies involving the amalgamation of non-thermal processes and infrared drying (Fig. 2C) is a feasible technique, as it; 1. reduced the energy cost (Boateng & Yang, 2021b), 2. improved the quality of dried GBS (lower color change, lower enzyme activity, high sensory scores, and improved texture) and physicochemical properties (Boateng, Zhang, et al., 2021). Sensory analysis, a scientific approach that uses the senses of smell, sight, taste, touch, and hearing to elicit,

measure, evaluate, and interpret product responses (Craine, Bramwell, Ross, Fisk, & Murphy, 2021; Ross, 2021), was also applied and the osmosonication (ultrasound pretreatment followed by infrared drying) had the highest sensory attributes (Boateng, Zhang, et al., 2021). Besides, osmosonication (Fig. 2C) had a slight microstructure deformation and the lowest water activity among the various hybrid treatments. Therefore osmosonication is a promising hybrid technology for GBS's processing. Comparing the pros and cons of the various thermal processing methods, it can be seen that the hybrid technology can improve the safety and stability of GBS along with the retention of texture and sensory properties. Nonetheless, more research is needed to improve osmosonication conditions.

Comparing the advantages and disadvantages of various technologies, it can be seen that hybrid technology (non-thermal pretreatment followed by drying) and MST's benefits far outweigh the other technologies. Nevertheless, further research is required through multi-response optimization to improve the physicochemical properties.

## 8. Effect of current technologies on the ginkgo seed's pharmacological activities

Natural products and therapeutic plants have sparked interest in thermal processing methods. Nonetheless, except a few medicinal plants, the effects of heat processing and their underlying causes are yet to be fully understood pharmacologically. Many recent investigations have found that the liberation of different compounds during the thermal processing of agro products increases biological activity (Hong et al., 2017; Jing, Zhou, Wang, Yin, & Zhang, 2021; Lim & Kim, 2018a). Besides, metabolites' content changes (e.g., antioxidants, phenolic) can occur because of processing (Alolga et al., 2021; Boateng & Yang, 2021d; Dzah et al., 2021). Thus, both the development of health-promoting constituents and potentially harmful health compounds may be affected by processing. Hence, there is also the need to review the aforementioned technologies on the ginkgo seed's pharmacological activities.

Polyphenolic compounds have been associated with anti-oxidants and anti-cancer activities, therefore maintaining polyphenols during processing is essential (Alolga et al., 2021; Boateng, Yang, & Li, 2020; Dzah et al., 2021). As shown in Table 3 and Fig. S5A, and Fig. S6, it can be observed that phenolic content can increase or decrease depending on the type of technology applied. Lactic acid fermentation has been used to improve the phenolic content of ginkgo products (Fig. S5A). A critical characteristic of lactic acid bacteria (LAB) is the bioconversion and biosynthesis of certain compounds that positively influence human health. Total phenolic content (TPC) increased considerably within 24 h of fermentation (Fig. S5A) in a study by Wang et al. (2019). After 24 h, there was a negligible reduction. The total phenolic content of GBS juices fermented by different strains was roughly 9% greater than that of unfermented GBS juices at the end of fermentation. Nevertheless, HPLC analysis of individual compounds showed degradation of some phenolic compounds such as ferulic acid, *p*-coumaric acid, and chlorogenic acid (CA). According to Wang et al. (2019), the decrease in CA may be linked to enzymatic degradation during LAB fermentation, and the degradation of CA led to an increase in ferulic acid and caffeic acid, which can be transformed by lactobacillus into vanillic acid, 4-vinyl guaiacol, and trace amounts of vanillin. Thus, although the TPC of the fermented ginkgo increased, certain individual phenolic compounds decreased due to various LAB activities. Boiling and steaming of GBS led to reduced total phenolic content (TPC) (Hong et al., 2017). In research by Hong et al. (2017), they found out that TPC was reduced as the boiling time increased (especially when it was boiled for >25 min), and boiling for 45 min had the highest phenolic degradation. This phenomenon was also observed for steaming. Hong et al. (2017) explained that the phenolic degradation during steaming and boiling might be due to the leaching of phenolic compounds into the water medium. However, it was observed that pan-frying had an increase or decrease in TPC depending on the

**Table 3**  
Influence of various technologies on ginkgo seed's pharmacological activities.

| Technology                                                                        | Conditions                                                                                                                                      | Compounds tested | Results                                                                                                                                                                                                                                                                             | Reference                       |
|-----------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------|------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------|
| Fermentation using <i>Bacillus natto</i>                                          | Stirring rate = 150 rpm, T = 36.4 °C, particle size = 60 mesh, pH = 6.5, t = 43 h, beads-solid ratio = 1 g/g and liquid–solid ratio = 16.4 mL/g | Flavonoids       | After the fermentation, the ginkgo seed's TFC reached $2.58 \pm 0.18$ mg RE /g. This is a 2.5-fold increase compared to fresh GBS.                                                                                                                                                  | Guo et al. (2018)               |
| Solid-state fermentation using <i>Bacillus natto</i>                              | Inoculation = $1 \times 10^7$ fungi/ 5 g residues, maltose and peptone = 2% w/v, pH = 7, moisture content = 40%, T = 25 °C, and time = 4 days.  | Flavonoids       | For quercetin, kaempferide, and isorhamnetin in fresh GBS were 0.18, 0.19, and 0.12 mg correspondingly. After fermentation, the quercetin, kaempferol, and isorhamnetin increased to 0.32, 0.37, and 0.23, correspondingly.                                                         | Guo et al. (2019)               |
|                                                                                   |                                                                                                                                                 | Antioxidants     | Fermented GBS exhibited a higher Fe <sup>2+</sup> chelating ability than fresh GBS.                                                                                                                                                                                                 |                                 |
| Fermentation using different LAB                                                  | A 300 mL of GBS juice was inoculated with 2% (v/v) inoculum and fermented for 4–48 h.                                                           | Antioxidants     | The LAB significantly enhanced the antioxidant                                                                                                                                                                                                                                      | Wang et al. (2019)              |
|                                                                                   |                                                                                                                                                 | Ginkgolides      | Terpene lactones in fermented GBS were 1.6-fold higher than fresh GBS.                                                                                                                                                                                                              |                                 |
| Boiling                                                                           | Boiled for 5, 15, 25, 35, and 45 min                                                                                                            | Phenolics        | The TPC was reduced as the cooking time increased. Boiling for 45 min had the lowest TPC.                                                                                                                                                                                           | Hong et al. (2017)              |
|                                                                                   |                                                                                                                                                 | Flavonoids       | The TFC was reduced as the cooking time increased. Boiling for 45 min had the lowest TFC.                                                                                                                                                                                           |                                 |
|                                                                                   |                                                                                                                                                 | Antioxidants     | The antioxidant activity (% DPPH) reduced as the cooking time increased. Boiling for 45 min had the highest reduction of antioxidant activity.                                                                                                                                      |                                 |
| Steaming                                                                          | Steamed for 10, 30, 50, 70 and 90 min                                                                                                           | Phenolics        | The TPC was reduced as the steaming g time increased. Steaming for 90 min had the lowest TPC.                                                                                                                                                                                       | Hong et al. (2017)              |
|                                                                                   |                                                                                                                                                 | Flavonoids       | The TFC was reduced as the steaming g time increased. Steaming for 90 min had the highest flavonoid degradation                                                                                                                                                                     |                                 |
|                                                                                   |                                                                                                                                                 | Antioxidants     | Steaming for 10, 30, and 50 min had a lower antioxidant (% DPPH) compared to the fresh GBS. However, the antioxidant activity increased when seamed for 70 and 90 min. Steaming for 90 min had the highest antioxidant activity                                                     |                                 |
| Pan-frying                                                                        | Pan-fried for 8, 11, 14, 17, and 20 min                                                                                                         | Phenolics        | The TPC increased when pan-fried for 8 and 11 mins. However, the TPC decreased after 11 min as the frying time increased. Pan-frying for 20 min had the highest degradation of phenolics.                                                                                           | Hong et al. (2017)              |
|                                                                                   |                                                                                                                                                 | Flavonoids       | The TFC increased when pan-fried for 8 and 14 mins. However, the TFC decreased after 14 min as the frying time increased. Pan-frying for 20 min had the highest flavonoid degradation.                                                                                              |                                 |
|                                                                                   |                                                                                                                                                 | Antioxidants     | The antioxidant activity increased from 8 to 14 min of pan-frying, then decreased at 17 and 20 min compared to the fresh GBS.                                                                                                                                                       |                                 |
| Roasting                                                                          | Time (min) and Temperature (°C)<br>150 °C for 15, 30, 45, and 60 min<br>180 °C for 15, 30, 45, and 60 min<br>210 °C for 15, 30, 45, and 60 min  | Phenolics        | The TPC in the 210 °C groups rose considerably with time ( $p < 0.05$ ). In the 150 and 180 °C heat treatment groups, however, there was no significant change ( $p > 0.05$ ). Compared to fresh GBS (5.71 mg GAE/g), the TPC increased in the 210 °C (9.83 mg GAE/g) after 60 min. | Lim & Kim (2018b)               |
|                                                                                   |                                                                                                                                                 | Antioxidants     | The DPPH and ABTS radicals increased with increasing temperature and duration of the heat treatment compared to the fresh. The 210 °C for 60 min had the highest antioxidant activity.                                                                                              |                                 |
| Fermentation with various rice wine starters (17 varieties of rice wine starters) | The rice wine starter volume, fermentation temperature, and time were 5% (w/w), 32 °C, and 48 hr, respectively.                                 | Flavonoids       | quercetin increased by 188.1%                                                                                                                                                                                                                                                       | Shen et al. (2020)              |
|                                                                                   |                                                                                                                                                 | Antioxidants     | Antioxidant (% DPPH) increased from 4.69 to 12.43 mg TE/g                                                                                                                                                                                                                           |                                 |
| Fermentation with rice wine starter (fermenting rice noodles and Rhizopus)        | T = 27°C, inoculum volume 7.6%, and t = 46 h                                                                                                    | Flavonoids       | The TFC of fermented GBS reached 0.832 mg RE/g (3.6 times that fresh GBS \.                                                                                                                                                                                                         | Shen et al. (2021)              |
|                                                                                   |                                                                                                                                                 | Antioxidant      | The antioxidant capacity of the fermented was 3 times that of fresh GBS.                                                                                                                                                                                                            |                                 |
| Submerged Fermentation using <i>Eurotium cristatum</i>                            | pH = 5.8, volue of medium = 100 mL<br>inoculum size = $5.1 \times 10^6$ CFU/ mL, and fermentation days = 4                                      | Flavonoids       | The TFC of the fermented GBS was almost the same as the fresh GBS ( $p > 0.05$ ).                                                                                                                                                                                                   | Zou, Zhang, Dong, et al. (2021) |
|                                                                                   |                                                                                                                                                 | Antioxidants     | The overall antioxidant value increased steadily during the fermentation process, peaking on the 5th day.                                                                                                                                                                           |                                 |
|                                                                                   |                                                                                                                                                 | Ginkgolides      | The terpene trilactones of the fermented GBS were almost the same as the fresh GBS ( $p > 0.05$ ).                                                                                                                                                                                  |                                 |
| Combined endogenous enzymatic hydrolysis with resin adsorption                    | Endogenous enzyme(s) at T = 40 °C, pH = 5.0 and t = 180 min.                                                                                    | Flavonoids       | The TFC of the processed GBS was almost the same as the fresh GBS ( $p > 0.05$ ).                                                                                                                                                                                                   | Zhang et al. (2020)             |
|                                                                                   |                                                                                                                                                 | Ginkgolides      | The terpene trilactones in processed GBS were 40% lower than fresh GBS.                                                                                                                                                                                                             |                                 |
| Solid-state Fermentation using <i>Eurotium cristatum</i>                          | pH = 5.0, CFU/ g of inoculum size = $2 \times 10^8$ , sterilized water = 3 mL, fermentation days = 4                                            | Flavonoids       | The TFC of the processed GBS was almost the same as the fresh GBS ( $p > 0.05$ ).                                                                                                                                                                                                   | Zou, Cao, et al. (2021)         |
|                                                                                   |                                                                                                                                                 | Antioxidants     | The antioxidant activity increased on the 4th day of fermentation.                                                                                                                                                                                                                  |                                 |
|                                                                                   |                                                                                                                                                 | Ginkgolides      |                                                                                                                                                                                                                                                                                     |                                 |

(continued on next page)

Table 3 (continued)

| Technology                                      | Conditions                                                                                                                                                                                                                                                                                                              | Compounds tested                                       | Results                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | Reference                            |
|-------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------|
| Membrane separation technology                  | Ultrafiltration membrane with MW of with 10 kDa: 0.2–0.3 MPa with a permeate volume (4.7 L)<br>Nanofiltration membrane with MW of with 0.6 kDa: 0.4–0.5 MPa with a permeate volume = 3 L                                                                                                                                | Flavonoids<br>Ginkgolides                              | The terpene trilactones of the processed GBS were almost the same as the fresh GBS ( $p > 0.05$ ).<br>The TFC of the processed GBS was almost the same as the fresh GBS ( $p > 0.05$ ).<br>The terpene trilactones in the processed GBS decreased by 43.6%.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               | Zou, Zhang, Wu, et al. (2021)        |
| Drying                                          | PVD (T = 80 °C VP/AP time = 10: 5)<br>HAD (T = 80 °C AV = 2 m/ s.)<br>IRD (T = 80 °C AV = 2 m/ s and H = 6 cm)<br>FD (Initially frozen (–20 °C, 24 h), and freeze-dried at T = –50 °C)                                                                                                                                  | Phenolics<br>Flavonoids<br>Antioxidants<br>Ginkgolides | The IRD treatment increased the TPC. The FD preserved the TPC, whereas the HAD and PVD lower TPC compared to the fresh GBS<br>When compared to fresh GBS, the processed GBS had a higher TFC. In addition, TFC was higher in heat-dried samples (IRD, HAD, and PVD) than in FD products.<br>The IRD recorded the highest antioxidant ability, and HAD had the least.<br>The ginkgolides decreased all the processed GBS compared to the fresh. For FD and IRD, ginkgolide A and C were found, however for HD and PVD, no ginkgolide A or C was found.                                                                                                                                                                                                                                                                                                                                                     | Boateng & Yang (2020)                |
| Drying followed by rehydration (60 °C in water) | HAD-R, IRD-R, PVD-R and FD-R                                                                                                                                                                                                                                                                                            | Phenolics<br>Flavonoids<br>Antioxidants                | The TPC of the rehydrated GBSs were greater than fresh GBS. The IRD-R had the TPC among the rehydrated GBS. The TFC showed a 100% increase in rehydrated products compared to the fresh GBS. The IRD-R recorded the highest TFC among the rehydrated products<br>The FRAP, ABTS, and CUPRAC were higher in rehydrated products than fresh GBS.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            | Boateng, Yang, Tahany, et al. (2020) |
| Optimizing IRD                                  | T = 80 °C, t = 159.68 min, and H = 12 cm                                                                                                                                                                                                                                                                                | Phenolics<br>Flavonoids<br>Antioxidants                | The TPC in fresh GBS was 4.76 mg GAE/g, while the dried GBS was 40 mg GAE/g.<br>The TFC increased from 0.82 mg RE/g in fresh GBS to 1.98 mg RE/g in the processed GBS.<br>When compared to fresh GBS, dried GBS had higher antioxidant activity. The antioxidant activity of the DPPH assay increased by >200%, whereas the anti-oxidant activity of ABTS increased by 17%.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               | Boateng, Yang, & Li (2020)           |
| Nonthermal pretreatment followed by IRD         | Control (no pretreatment)<br>OS : sucrose 15% w/v, T = 30 °C, t = 30 min<br>OS + V: sucrose 15% w/v, 30C, VP/AP time = 10: 5 t = 30 min<br>US: PO:PF time = 2:10, t = 30 °C, PD = 100 W/l, F = 28 kHz and t = 30 min<br>OS + US: sucrose 15% w/v, PO:PF time = 2:10, t = 30 °C, PD = 100 W/l, F = 28 kHz and t = 30 min | Phenolics<br>Flavonoids<br>Antioxidants<br>Ginkgolides | Compared to fresh GBS, non-thermal pretreatment and control (no pretreatment before infrared drying) increased TPC. However, when comparing the control and pretreatment GBSs' TPC, the osmotically treated GBS (OS, OS + V, and US + OS) had a lower TPC than the control and US.<br>All flavonol aglycones components and TFC had the lowest values in the OS. In terms of TFC, there was no significant difference between the OS + V, US, and US + OS and the control.<br>The control group showed the highest antioxidant activity (FRAP, DPPH, and ORAC) compared to the other pretreatments. In addition, as compared to osmotic treatments, the US had the maximum antioxidant activity of all the pretreated GBS.<br>The ginkgolides were reduced during the various treatments compared to the fresh GBS. The ginkgolide B and C degradation rates faster in the pretreatments than in control. | Boateng & Yang (2021a)               |

**Note** LAB, lactic acid bacteria; HAD, hot-air drying; PVD, pulsed-vacuum drying; FD, freeze-drying; IRD, infrared drying; FD-R, freeze-dried rehydration; IRD-R, infrared-dried rehydration; HAD-R, hot-air dried rehydration; PVD-R, pulse-vacuum dried rehydration; OS, osmotic; OS + V, osmotic vacuum; US, ultrasound; US + OSS, osmosonication pretreatment followed by infrared drying; VP: AP, vacuum and atmospheric pressure ratio; T, temperature; t, time; CFU, colony-forming units; PD, power density; PO: PF, the ratio of pulsed-on to pulsed-off time; MW, molecular weight; LAB, lactic acid bacteria; GA, ginkgolic acid.; TPC, total phenolic content; TFC, total flavonoids content; DPPH, 1,2-diphenylpicrylhydrazyl; ABTS, 2,2-azino-bis (3-ethylbenzothiazole-6-sulfonic acid); FRAP, ferric reducing antioxidant power, RE, Rutin equivalent; GAE, gallic acid equivalent; ORAC, Oxygen radical absorbance capacity.

processing time. There was a slight increase at 8 and 11 min, but no significant difference was observed. However, there was a significant decrease after 14 min to 20 min ( $p < 0.05$ ). Besides, the pan-frying had the lowest phenolic degradation compared to steaming and boiling. This shows that different cooking methods affect GBS's pharmacological activities. Drying is a critical processing step for extending shelf life (Alolga et al., 2021). Studies have shown that drying of GBS (Table 3) can have negative, neutral, and positive effects on phenolics (Boateng, Soetanto, Yang, et al., 2021; Boateng & Yang, 2020; Boateng, Yang, Tahany, et al., 2020). In the research by Boateng & Yang (2020), they observed infrared-drying (IRD) increased the TPC, the freeze-drying

(FD) preserved the TPC, whereas the hot-air drying (HAD) and pulsed-vacuum drying (PVD) lowered TPC compared to the fresh GBS. According to Boateng & Yang (2020), the increase in TPC for infrared-dried GBS could be due to the conversion of a large number of polymeric polyphenols initially bonded to oligosaccharides and polysaccharides into free polyphenols (low MW phenolic compounds) during heating due to infrared radiation's high penetration ability. This was also confirmed in a phytochemical profile analysis using gas-chromatography mass-spectrometry (GC–MS/MS) (Boateng & Yang, 2021c). As a result, IRD can break apart more cells and liberate free polyphenols. Again, Boateng & Yang (2020) explained that enzymes

become inactive when freeze-dried. Nonetheless, when the GBS is removed from the dryer, the degrading enzyme may still be active, and polyphenol oxidase converts polyphenols to quinones. Hybrid technology (drying followed by rehydration) has revealed an increase in phenolic content after processing (Table 3). In a study by Boateng, Yang, Tahany, et al. (2020), they observed that the various rehydrated GBS had higher TPC values than fresh GBS. The IRD-R (infrared drying followed by rehydration) recorded the highest TPC among the rehydrated samples. This shows the hybrid technology had a significant effect on TPC. To further understand the mechanism of phenolic increase in the IRD, a response surface methodology (RSM) was applied (Boateng, Yang, & Li, 2020) in visualizing the relationship between independent factors and TPC (Fig. S6). The interactive effect of temperature and height significantly impacted TPC (Fig. S6A). The TPC rapidly increased as the temperature and height of the infrared emitter increased; thus, an increase in TPC with respect to temperature, time, and height of the infrared emitter (Fig. S6A-C) could be due to the conversion of a significant amount of polyphenols that were initially strongly bonded to oligosaccharides and polysaccharides into free polyphenols (via esterification reaction) during heating. Boateng & Yang (2021a) applied hybrid technologies (integrated non-thermal pretreatments and drying) to study the effect of this technology on phenolics (Fig. 2C). Their results (Table 3) showed that the osmotically-treated GBS (osmotic, osmovacuum, and osmosonication) had a lower TPC ( $p < 0.05$ ) while the control (no pretreatment before drying) and sonication (GBS immersed in distilled water) had the highest TPC with no significant difference ( $p > 0.05$ ). According to Boateng & Yang (2021a), the lowest TPC values for osmotically-treated GBS could be due to increased mass transfer between GBS and the osmotic medium, which may benefit water loss, but adversely affect phenolics due to increased migration throughout osmotic dehydration and promoting the leaching of some phenolics. Hence, the technologies applied in GBS processing can decrease, maintain, or reduce phenolics.

Flavonoids are the largest unit of phenolic plants, accounting for more than half of the eight thousand phenolic compounds that occur naturally (Boateng & Yang, 2021d; Dzah et al., 2021; Guo et al., 2018). There is currently a growing interest in flavonoid studies due to the possibility of improving public health through diet, where the consumption of fruit and vegetables can improve one's health (Dzah et al., 2021; W. Liu et al., 2021; Zou, Zhang, Dong, et al., 2021). Flavonoid-rich fruits, nuts, and vegetables can be eaten as either fresh or processed foods. Nevertheless, various processing techniques can positively or negatively affect flavonoids in GBS, as shown in Table 3. Fermentation of GBS can have a positive or neutral effect on the flavonoid content. In research by Guo et al. (2018) using *Bacillus natto* for GBS fermentation, they observed that after the fermentation, the ginkgo seed's TFC reached 2.58 mg RE /g. This was 2.5-fold higher than fresh GBS. Guo et al. (2018) explained that the magnetically immobilized *Bacillus natto* promoted the release of flavonoids during GBS fermentation. In a solid-state fermentation using *Bacillus natto*, as shown in Table 3, Guo et al. (2019) observed that quercetin, kaempferol, and isorhamnetin (the three major flavonoid aglycones) in fresh GBS were 0.18, 0.19, and 0.12 mg, correspondingly. After fermentation, quercetin, kaempferol, and isorhamnetin increased to 0.32, 0.37, and 0.23, correspondingly. *Bacillus subtilis* strains can synthesize complex enzymes, including cellulase and  $\beta$ -glucosidase, to enhance the release of total phenolics and flavonoids during fermentation (Guo et al., 2019). As a result, the GBS's total flavonoid content increased after the fermentation. Shen et al. (2021) fermented GBS with a rice wine starter (fermenting rice noodles and *Rhizopus*), as shown in Table 3. The TFC of fermented GBS reached 0.832 mg RE/g (about 3.6 times that of fresh GBS). However, submerged fermentation using *Eurotium cristatum* (Zou, Zhang, Dong, et al., 2021), and solid-state fermentation using *Eurotium cristatum* (Zou, Cao, et al., 2021), showed that the TFC of the processed GBS was almost the same as the fresh GBS ( $p > 0.05$ ). This indicates that the fermentation technology and the processing conditions applied can have a neutral or positive

effect on GBS; thus, it must be considered. Moreover, various literature has shown that thermal processing can have a negative or positive impact on the flavonoids depending on the processing method employed (Boateng & Yang, 2020; Boateng, Yang, & Li, 2020; Boateng, Yang, Tahany, et al., 2020; Hong et al., 2017). In research by Hong et al. (2017), the TFC reduced as the boiling time increased, and boiling for 45 min had the lowest TFC. A similar phenomenon was observed for steaming as TFC was reduced when the steaming time increased, and 90 min of steaming had the highest flavonoid degradation. However, for pan-frying, TFC increased when GBS was pan-fried for 8 and 14 mins and decreased after 14 min. Drying and hybrid technology (drying followed by rehydration, nonthermal pretreatment followed by infrared drying) can increase flavonoids (Boateng & Yang, 2021a; Boateng, Yang, & Li, 2020; Boateng, Yang, Tahany, et al., 2020). As shown in Table 3, there was an increase in TFC for the processed GBS compared to the fresh, showing that drying increases the TFC in GBS. Heat-dried samples (IRD, HAD, and PVD) had higher TFC than FD products. Boateng & Yang (2020) explained that the higher TFC in the heat-dried GBS than the freeze-dried GBS might be due to the high temperature that cleaves glycosidic bonds to release bound flavonoids. Thus, the above review on the effect of technologies can have a positive, neutral or negative impact on TFC; therefore, it must be considered in the technology selection process.

The synergy between two very distinct chemical groups, terpene trilactones (ginkgolides and bilobalide) and flavonoids, gives ginkgo its pharmacological importance (Boateng & Yang, 2020, 2021a; Le et al., 2017; Wang & Zhang, 2019). Terpene trilactones (TTL) are distinct components of GB, and the three distinct forms (ginkgolide A, B, and C) have different pharmacological activity modes. The TTLs, particularly ginkgolide B, primarily act as antagonists of platelet-activating factor receptors, which are used to treat neurological and inflammatory diseases. At the same time, Ginkgolide C has a variety of biological functions, ranging from platelet reduction to Alzheimer's disease treatment (Boateng & Yang, 2022; Le et al., 2017). As shown in Table 3, it can be observed that ginkgolides (terpene lactones) can increase or decrease depending on the type of technology applied. In research by Wang et al. (2019) using lactic acid bacteria (LAB) fermentation, the ginkgolides A, B, and C increased significantly during LAB fermentation (increased as the fermentation time increased), especially ginkgolide C. After 48 h of fermentation, the TTL in GB kernel juice increased by 65.56%, 59.01% and 63.89% for *Lactobacillus acidophilus*, *Lactobacillus plantarum*, and *Lactobacillus casei* fermentation, respectively. However, it must be noted that not all fermentation increase the ginkgolides as research shows that the kind of fermentation and the microorganism used can improve or maintain the ginkgolides. For example, in submerged fermentation using *Eurotium cristatum*, Zou, Zhang, Dong, et al. (2021) reported that terpene trilactones of the fermented GBS were almost the same as the fresh GBS ( $p > 0.05$ ). In a solid-state fermentation using *Eurotium cristatum*, Zou, Cao, et al. (2021) also reported that the terpene trilactones of the processed GBS were almost the same as the fresh GBS ( $p > 0.05$ ). For the degradation of ginkgolides in processed GBS, various works of literature have shown thermal processing (Boateng & Yang, 2020, 2021a) and adsorption technology (Zhang et al., 2020; Zou, Zhang, Wu, et al., 2021) reduced the ginkgolides. The research by Zhang et al. (2020) using combined endogenous enzymatic hydrolysis with resin adsorption (Table 3) reported that the terpene trilactones in processed GBS were 40% lower than in fresh GBS. In a membrane separation technology by Zou, Zhang, Wu, et al. (2021), the terpene trilactones in the processed GBS decreased by 43.6%. Zou, Zhang, Wu, et al. (2021) explained that the molecular weights of terpene trilactones are 400 Da. Thus they could penetrate the nanofiltration membrane leading to lower terpene trilactones in GBS. Likewise, drying (Boateng & Yang, 2020), combined hybrid drying technology (integrated non-thermal pretreatment and drying) (Boateng & Yang, 2021a), frying, boiling, and steaming (Zhang, Zhang, Li, Meng, & Liang, 2015) reduced the ginkgolides. A study by Boateng & Yang (2021a) using various integrated

non-thermal pretreatment and drying (Table 3) reported that the ginkgolides declined during the various treatments. Compared to the control, ginkgolide B and C's degradation rates were higher in the pretreatments (Table 3). Ginkgolide B and C leached into the liquid media (during pretreatment), reducing their content, according to Boateng & Yang (2021a). Another cause could be the effect of pretreatment followed by drying, which synergistically reduced ginkgolide. The lowest ginkgolide B and C levels were found in the osmotic vacuum pretreatment, whereas the highest amount was found in the ultrasound pretreatment. This demonstrates that different pretreatments have varying effects on the rate of ginkgolide degradation. Similarly, Boateng & Yang (2021a) findings were in consonance with Zhang et al. (2015), who boiled, steamed, and fried GBS, as well as Boateng & Yang (2020), who applied different drying techniques (Table 3). All these works of literature have revealed that fermentation technology could have a favorable, neutral impact on ginkgolides, whereas thermal processing and adsorption technology can reduce the ginkgolides in the processed GBS.

Several studies have revealed a link between antioxidant-rich food and good health, implying that eating foods rich in antioxidants can help preserve health and prevent certain diseases (Alasalvar, Salvadó, & Ros, 2020). Current findings (Table 3, Fig. S5A and B) showed antioxidant activity in processed GBS can increase or decrease. In a report by Hong et al. (2017), the antioxidant activity of boiled GBS reduced as the boiling time increased. Nevertheless, antioxidant activity increased from 8 to 14 min of pan-frying, then decreased at 17 and 20 min compared to the fresh GBS. In research by Boateng & Yang (2020), the antioxidant of the processed GBS was higher than the fresh GBS due to the drying process that might have led to the active ingredients bonded to the tissues being released, leading to an antioxidant effect. In the hybrid technology (drying followed by rehydration) by Boateng, Yang, Tahany, et al. (2020), they observed that the antioxidant activities were higher in rehydrated products than fresh GBS. Likewise, in research by Lim & Kim (2018b) using roasting as heat treatment, as shown in Table 3, they observed that the antioxidant activity increased with increasing temperature and duration of the heat treatment compared to the fresh. Nevertheless, in a nonthermal pretreatment followed by infrared drying, the control group (no pretreatment before drying) had the highest antioxidant activities compared with the various pretreatments. This indicates that although antioxidant activity increase during drying, when the GBS are pretreated before drying, the antioxidant activity is lower than the control (no pretreatment before drying) due to higher migration of compounds that caused the leaching of some bioactive compounds during pretreatment; as a result, a decreased antioxidant activity.

Notwithstanding, when it comes to fermentation, it was observed that irrespective of the microorganism used or the type of fermentation, the antioxidant activity of the fermented product increased (Table 3, Fig. S6 B-C). In research by Wang et al. (2019) using lactic acid bacteria (LAB) fermentation, antioxidant activities were enhanced continuously throughout the entire fermentation (Fig. S6 B-C). After 48 h of fermentation, the antioxidant activity increased from 11.18 to 15.13, 15.26, and 15.63 mmol Trolox/L for the juices incubated with *Lactobacillus acidophilus*, *Lactobacillus plantarum*, and *Lactobacillus casei*, correspondingly. According to Wang et al. (2019), the increase in aglycone isoflavone and TPC can enhance antioxidant activity because these antioxidants have been proven to be involved in the termination of free radical reactions; thus, phenolic accumulation is an essential contributor to increasing antioxidant ability. Similarly, in a solid-state fermentation using *Bacillus natto*, Guo et al. (2019) witnessed that the fermented GBS exhibited a higher  $\text{Fe}^{2+}$  chelating ability than fresh GBS. Shen et al. (2020) reported an increased antioxidant capacity from 4.69 to 12.43 mg TE/g in a fermented GBS. In a submerged fermentation using *Eurotium cristatum*, the total antioxidant value kept increasing from the beginning of fermentation and peaked on the fifth day (Zou, Zhang, Dong, et al., 2021). These findings revealed that various technologies could have a negative or positive effect on the antioxidant

potential of processed GBS. As a result, they must be considered when selecting the appropriate technology.

To sum up, drying and hybrid-drying technology increased the phenolic, flavonoids, and antioxidants; simultaneously, it reduced the ginkgolides. Lactic acid fermentation improved the phenolics, antioxidants, and ginkgolides, whereas adsorption technology reduced the ginkgolides, maintained the flavonoids, and increased the antioxidants in processed GBS.

## 9. Conclusion and future studies

In summary, the above review has shown that thermal treatment (boiling, frying, roasting, drying, microwaving, and steaming), fermentation (using various microorganisms and various fermentation methods), adsorption technology (resin adsorption, membrane separation), high-pressure processing, and hybrid technologies (drying followed by rehydration, non-thermal pretreatments followed by infrared drying, enzymatic hydrolysis followed by resin adsorption, high-pressure processing combined with enzymatic hydrolysis) can reduce toxic components in GBS. Furthermore, comparing the advantages and disadvantages of various technologies, it can be seen that osmosonication (hybrid technology) and membrane separation's (adsorption technology) benefits far outweigh the other technologies. Osmosonication 1. increased the phenolic, flavonoids, and antioxidants; 2. reduced energy consumption during processing 3. enhanced the safety and stability of GBS along with the retention of texture and sensory properties and 4. increased the shelf-life. The MST is also a feasible technology with 1. a potent removal of ginkgotoxin 2. higher solubility for the processed GBS, 3. Retention of flavonoids, and 4. Retention of freeze-thaw stability. This review brings to light the new possibilities of using osmosonication (hybrid technology) and membrane separation technology since they tend to reduce toxic compounds and liberate more bioactive compounds during GBS processing.

For future research, a study is required for osmosonication through multiple response optimization to retain other critical bioactive compounds like ginkgolides and ascorbic acid. Likewise, for MST, future research is needed to reduce the degradation of ginkgolides. Besides, the shelf-life of the processed GBS is of great economic importance. Thus further research on how MST could be merged with other technologies such as thermal, fermentation, and emerging technologies (ultrasound, pulse-electric field, or pulse-magnetic field technology) is required. In-vivo studies are needed to investigate the effects of the processed GBS on physiological and biochemical reactions in living organisms and the availability of large and coordinated databases. Research needs to be done about the GBS being used as a nutraceutical and exploring novel compounds applicable to the pharmaceutical and food industries since the toxic components have been reduced to a safe level. Intensive research on processed GBS's bioactivities and a thorough knowledge of the mechanism should be done.

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## Dedication

Dedicate this article to Mr. Oppong Mensuo, Mr. Opoku Nsafoah, and Mr. Christian Yeboah, who died in 2021. Peace be with them till we meet again.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foodchem.2022.132408>.

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