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Sprouting facilitates the antiglycative effect of black soybean (*Glycine max* (L.) Merr.) by promoting the accumulation of isoflavones

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ABSTRACT

The exposure of advanced glycation end products (AGEs) can induce chronic inflammation, oxidative stress, and accelerated aging, contributing the onset and progression of many diseases especially diabetic complications. Therefore, the searching of antiglycative foods is of practical significance, which may serve as a strategy in the attenuation of AGEs-associated diseases. In this study, we evaluated the antiglycative potential of some beans and bean sprouts that were common in our daily life. The results revealed that sprouting enhanced the antiglycative activity of beans, with black soybean sprouts demonstrating the highest efficacy (4.92-fold higher than the unsprouted beans). To assess practical implications, we examined the antiglycative activity of black soybean sprouts in pork soup, a popular food model that incorporates sprouts. Our findings confirmed the inhibitory effect on a dose-dependent manner. Through open column fractionation, we identified isoflavones and soyasaponin Bb as the candidates responsible for these effects. Additionally, compare to the unsprouted black soybeans, we found significant increases in the levels of antioxidative properties (2.51-fold), total phenolics (7.28-fold), isoflavones, and soyasaponin Bb during the sprouting process. Further studies determined that genistein, genistin, and daidzin were the major antiglycative compounds in black soybean sprouts. Collectively, this study emphasizes the benefits of sprouted beans and offers foundation for the development of functional sprouting foods.

1. Introduction

Beans such as soybean (*Glycine max*), mung bean (*Vigna radiata* L.), and pea (*Pisum sativum*), have been consumed by humans for centuries. These beans are high in nutritional value, containing abundant proteins, fibers, oils, and essential minerals and trace elements. They are also rich in bioactive compounds including polyphenols, peptides, and polysaccharides, which offer notable health benefits. Amongst, black soybean deserves special attentions. Black soybean is the mature seed of *Glycine* max (L.) Merr and has a significant role in both traditional Chinese cuisine and medical practices. Due to its high content of dietary fiber and functional components, black soybeans gained popularity in recent years and are often referred to as the king of beans, ascribed to their wide-ranging market prospects and therapeutic values (Kumar et al., 2022). Therefore, the study of black soybeans has garnered increasing public interests. For example, Kim et al. demonstrated that the anthocyanins present in black soybean coats exhibited anti-obesity activity by reducing food intake and fat accumulation (S. Y. Kim et al., 2015). Yamashita et al. have discovered that black soybeans can improve blood vessel function, reduce blood pressure, and provide protective effects against cardiovascular diseases (Yamashita et al., 2020). In particular, these effects are attributed to the activation 5′ AMP-activated protein kinase (AMPK) and glucose transporter 4 (GLUT4) signaling pathways by black soybean proteins. Such activation was mainly caused by the phosphorylation of AMPK. Active compounds in black soybean seed coat extract (e.g. cyanidin-3-glucoside and

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procyanidins) was shown to dose-dependently increased phosphorylation of liver kinase B1 (LKB1, an upstream kinase for AMPK phosphorylation) (Hironao et al., 2020), then increase AMPK phosphorylation significantly in the liver, skeletal muscle and adipose tissues *in vivo* and *in vitro*. Moreover, the upregulated GLUT4 translocation was induced by phosphorylation of AMPK directly, or through a series of regulatory processes involving the upregulation of glucagon-like peptide (GLP-1) and insulin secretion (Yamashita et al., 2020; Yamashita et al., 2020). Besides, anthocyanins in black soybeans showed anti-inflammatory and anti-diabetic effects in the coculture of hypertrophied 3T3-L1 adipocytes and RAW 264.7 macrophages (J. N. Kim et al., 2021). Other therapeutic benefits like anti-oxidant, anti-cancer, and neuroprotective activity of black soybeans have been explored with extensive evaluation and validation (Chen et al., 2019; J. Kim et al., 2020; Shabbir et al., 2022). Noteworthy, black soybeans and soybeans share similar nutritional compositions, but black soybeans distinguished themselves by containing higher levels of anthocyanins (e.g., centaurin-3-galactoside, centaurin-3-glucoside, and delphinus-3-glucoside) and soyasaponins. These bioactive compounds contribute to the stronger antioxidant and anti-aging effects of black soybeans compared to regular soybeans (R. Zhou et al., 2017). Except that, black soybeans might offer other potential benefits that warrant further explorations.

The existence of anti-nutrients such as protease inhibitors, lectins, and phytic acid, has greatly limited the dietary application of beans. To address this issue, various approaches have been developed to destroy or reduce these anti-nutritional factors, including traditional thermal processing, fermentation, and the emerging practice of sprouting. Among these approaches, sprouting has been recognized as the most promising and effective strategy to enhance the nutritional value of beans and decrease the content of anti-nutritional factors (Gu et al., 2017). Sprouts are a type of novel vegetable that produced through seed germination. They have largely gained popularity among consumers due to their edibility, short growth cycle, low cost, and rich profile of bioactive substances (Waliat et al., 2023). During the sprouting process, there is a notable increase in protein content (by ~ 50%), free amino acids (by ~ 3 fold), isoflavones, and vitamins, while the oil and fatty acid content decreases (Ghani et al., 2016). Sprouts are deeply welcomed by consumers. Currently, a wide variety of bean sprouts are sold in the market, with black soybean sprouts, soybean sprouts, mung bean sprouts, cowpea (*Vigna unguiculata*) sprouts, and pea sprouts being the most prevalent ones. During the sprouting process, the nutrients and bioactive ingredients in the beans undergo transformations. On one hand, the high molecular storage components (e.g., proteins and polysaccharides) are converted into soluble small molecule compounds (e.g., free amino acids, glucose, and inositol) that are easily absorbed by human body. It also enriches the content of trace elements such as iron, manganese, and zinc, which are crucial for maintaining human health (Ebert, 2022; Wang et al., 2022). On the other hand, the content of certain bioactive compounds (e.g., saponins) also increase significantly (G. Huang et al., 2017). For instance, the total saponins content in soybeans showed a dose-time dependent increase with the duration of sprouting, with soyasaponin Bb increased by more than 9 times compared to the initial seeds on the 5th day after sprouting (Chang and Han, 2016). Moreover, these bean sprouts have been demonstrated to possess multiple beneficial effects, including antioxidant, anti-inflammation, anti-obesity, cancer prevention, lowering blood lipids, and reducing the risk of cardiovascular complications (Aloo et al., 2021; Geng et al., 2022; Kathuria et al., 2023). Intriguingly, these bean sprouts have been found to be superior than beans in terms of their ability to provide higher level of health-promoting substances (X. Huang et al., 2014).

Advanced glycation end products (AGEs) are a heterogeneous group of glycated proteins or lipids that result from complex metabolic pathways. They are involved in the pathophysiology of various diseases, including diabetic complications, Alzheimer's disease, cancers, and liver cirrhosis (Koska et al., 2022; Lyu et al., 2023; Q. Zhou et al., 2020). Therefore, inhibiting the formation of AGEs is critical in the controlling of these chronic diseases and the foods with antiglycative properties serve as a practical and economic approach. In the past 50 years, significant efforts have been made in this field by dietary foods and food bioactive components. For instance, our previous work has identified the antiglycative effect of polyphenols in Hong Dou Shan (*Taxus chinensis*) leaf tea and apple (*Malus pumila* Mill) flower. However, limited work has been conducted to evaluate the antiglycative potential of bean sprouts, despite their assumed numerous benefits. We propose that the changes in these nutrients and compounds induced by sprouting may enhance the antiglycation effects. Therefore, the present study aims to contribute to this field by evaluating the antiglycative effects of bean sprouts compared to their initial beans, including black soybean, mung bean, grey pea, cowpea, and soybean. This assessment was conducted in both a model system (fructose-BSA model) and a real food system (meat-sprout soup). Additionally, the active components in the bean sprouts which had the highest antiglycative potential were identified through comprehensive chromatographic separation and analyses.

2. Methods and materials

2.1. Chemicals and reagents

All beans were purchased from Shangdong Shouhe Seeds Co., Ltd. (Weifang, China). Lean pork was obtained from a local Renrenle supermarket (Shenzhen, China). Bovine serum albumin (BSA), methylglyoxal (MGO; 40% aqueous solution), 5-methylquinoxaline (5-MQ), o-phenylenediamine (OPD), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and Sephadex LH20 were purchased from Sigma-Aldrich (St. Louis, MO, USA). Hydrochloric acid, fructose, ferrous chloride, ferrozine, Tween-20, sodium dodecyl sulfate (SDS), β-mercaptoethanol, and Tris-HCl were obtained from Macklin (Shanghai, China). The standard compounds of daidzin, genistin, daidzein, genistein, and soyasaponin Bb were bought from ChemFaces (Wuhan, China). HPLC grade and LC/MS grade acetonitrile (ACN) were bought from Xilong Scientific Co., Ltd. (Foshan, China). Analytical grade ethanol and methanol were sourced from Guangshi Reagent Technology Co., Ltd. (Zhaoqing, China). The ELASA AGE assay Kit was from Abcam (ab238539, Abcam Limited, Cambridge, UK). The Plant Phenol Assay Kit was bought from Solarbio (BC1345, Beijing Solarbio Science & Technology Co., Ltd, Beijing, China).

2.2. Cultivation of bean sprouts and sample extraction

Beans were soaked in water at 40 °C for 5 h and then transferred into a sprout planting machine named Caiduoduo Intelligent Hydroponic Planter (SPZW-A01WU2, Haier Group Corporation, Qingdao, China). The machine was set to a lighting time of 10 h per day, and tap water was added according to the supplier's guidelines to maintain a moist environment. Following that, 5 sprouts from each type of beans were harvested every 24 h to record their length by vernier caliper and their weight by precision balance. The total sprouting duration for the beans were 7 days (Cheng et al., 2023). After sprouting, 30 sprouts of each type were ground into small particles by a flour grinder (SMF2002, Zhejiang Supor Co. Ltd., Hangzhou, China). The ground sprouts were mixed with 100 mL of 95% ethanol, ultrasonic extracted at room temperature for 30 min, and filtered through a qualitative filter paper. The filtrate was collected and the residue underwent two additional extractions with 100 mL of 95% ethanol each time. All filtrates were combined and vacuum evaporated to dryness using a rotary evaporator under 40 °C. Then 10 mL methanol was added to re-dissolve the residue and stored at −80 °C until further use (Q. Zhou et al., 2021). Similarly, 30 unsprouted beans of each type was also extracted using the same method to enable appropriate comparisons.

2.3. Antiglycative analysis of beans and sprouts using BSA model

The fructose-BSA model was established based on our previous publication (Q. Zhou et al., 2023). In brief, a solution was prepared by dissolving 2.5 M fructose and 50 mg/mL BSA in 10 mM PBS. Then 1 mL of the prepared solution was added with 100 μL of each extracted sample, vortexed, and incubated in darkness at 37 °C for 7 days. Meanwhile, 100 μL methanol was added into 1 mL prepared solution to serve as a positive control and 100 μL methanol in 1 mL BSA (50 mg/mL) solution was used as a negative control. After incubation, 150 μL of each solution was transferred into a black 96-well plate. The fluorescence intensity was detected using a multimode plate reader (BioTek Synergy H1, Agilent Technologies Co. Ltd., Santa Clara, CA, USA) with excitation/emission wavelength at 370/440 nm. The formula for calculating AGEs inhibition rate was as follows:

$$AGEs\ inhibition\ rate\ (\%) = \left(1 - \frac{Ext}{Ctl}\right) \times 100\%$$

Ext: fluorescent intensity of each extract and Ctl: fluorescent intensity of the positive control.

2.4. Evaluation of RCS trapping ability of beans and bean sprouts

MGO was selected as the representative RCS. It serves as a pivotal indicator in assessing RCS trapping ability due to its high reactivity, well-established formation mechanisms, ubiquitous presence, and reliable detection methods, reflecting its significance in evaluating antiglycative strategies (Schalkwijk and Stehouwer, 2020). Each bean/sprout extract (100 μL) was mixed with 50 μL of 50 mM MGO, and then 850 μL PBS was added as the experimental group. 100 μL methanol was mixed with 50 μL of 50 mM MGO and 850 μL PBS as a positive control. 100 μL methanol mixed with 900 μL PBS was applied as a negative control. All samples were incubated at 37 °C for 24 h. After that, 5 μL of 500 mM OPD and 5 μL of 50 mM 5-MQ was added into the samples, followed by incubation for 2 h at 37 °C. The reaction was terminated by adding 5 μL perchloric acid. And then the samples were mixed, centrifuged, filtered through a 0.22 μm membrane, and loaded into HPLC system (Gao et al., 2020). The HPLC system was Waters 2695 and 2998 PDA Detector (Waters Corporation, Milford, MA, USA), equipped with a C_{18} reverse column (4.6 mm × 250 mm, 5 μm particle size; Alltima, Avantor Inc., Radnor, PA, USA). The mobile phase was 0.3% acetic acid and ACN with gradient elution from 35%~65% in 21 min. The detection wavelength was set at 316 nm, and the flow rate was 1 mL/min (Sun et al., 2019; Sun et al., 2019). In this analysis, the unreacted MGO was derivatized by OPD into 2-MQ and was calculated using the ratio of 2-MQ to 5-MQ (as an internal standard). The MGO inhibition rate of each extract to was calculated by the following formula:

$$MGO\ inhibition\ rate\ (\%) = \left(1 - \frac{Ext}{Ctl}\right) \times 100\%$$

Ext: the amount of MGO in solutions containing bean or bean sprout extracts and MGO (experimental group); Ctl: MGO content in the solution containing only MGO (positive control).

2.5. Evaluation of antioxidant activity in various bean and bean sprout extracts

The antioxidant activities of bean and bean sprout extracts were evaluated by DPPH clearance efficiency method (Gao et al., 2020). In the experimental group, 100 μL of each extract was added to 1 mL of 0.2 mM DPPH solution. In the blank control group, 100 μL methanol was added to 1 mL of 0.2 mM DPPH solution. All groups were incubated at 37 °C for 30 min. After that, 150 μL of the solution was transferred to a transparent 96 well plate, and the absorbance value at 517 nm was measured by the BioTek Synergy H1 multimode plate reader. The DPPH free radical scavenging rate was calculated by the following formula:

$$DPPH\ radical\ scavenging\ rate\ (\%) = \left(1 - \frac{Ext}{Ctl}\right) \times 100\%$$

Ext: $OD_{570\ nm}$ of each experimental group and Ctl: $OD_{570\ nm}$ of blank control group.

2.6. Evaluation of metal chelating activity in various bean and bean sprout extracts

The metal chelation capacity of bean and bean sprout extracts were detected according to the method of Ho et al. (Ho et al., 2014). In the experimental group, 100 μL extracts were mixed with 100 μL ferrozine (2 mM), 10 μL ferrous chloride (4 mM), and 790 μL deionized water to make a final volume of 1 mL. In the blank control group, 100 μL extracts were substituted by 100 μL methanol. All solutions were incubated at room temperature for 10 min and 10 μL of cooled perchloric acid was added to create an acidic environment and precipitate proteins. After that, the supernatant was centrifuged at 10000×g for 10 min at 25 °C. Then 150 μL supernatant was transferred to a transparent 96 well plate and the absorbance value at 562 nm was measured using a BioTek Synergy H1 multimode plate reader. The metal chelating activity of each extract was calculated using the following formula:

$$Metal\ Chelating\ activity\ (\%) = \left(1 - \frac{Ext}{Ctl}\right) \times 100\%$$

Ext: $OD_{562\ nm}$ of each experimental group and Ctl: $OD_{562\ nm}$ of blank control group.

2.7. Detection of total phenolic content in beans and bean sprouts

To determine the total phenolic content of black soybean sprout extracts from day 0 (the unsprouted seed) to day 7, a Plant Phenol Assay Kit was employed. In brief, 30 beans or 30 sprouts cultivated at designated days were ground into small particles and then ultrasonic extracted by 95% ethanol, following the method mentioned in 2.2. After that, the extracts were used for the determination of total phenolic contents. The experimental group was set up by mixing 10 μL extracts with 50 μL Reagent I. Meanwhile, the extracts were replaced by 10 μL deionized water in the control group. All groups were thoroughly vortexed for 2 min, added with 50 μL Reagent II, and 90 μL deionized water, left at room temperature for 10 min. Then 150 μL of the solution was transferred to a transparent 96-well plate. The absorbance value at 760 nm was measured. With reference to the standard curve, the total phenolic content of each bean/sprout was calculated using the following formula:

$$Total\ phenolic\ content = \frac{0.833y}{W}$$

y: concentration of each bean or bean sprout and W: weight of each bean or bean sprout.

2.8. Preparation of pork soup model

Lean pork was processed by a meat grinder (JR54S-U, Zhejiang Supor Co. Ltd., Hangzhou, China). 50 g ground pork was weighed and mixed with 250 mL deionized water. Additionally, 0 g, 15 g, 30 g, and 60 g black soybean sprouts collected on the 7th day of sprouting were added to different experimental groups. The mixture was then heated using the fine stew mode of a pot stew appliance (G55A, Shenzhen Buydeem Technology Co. Ltd., Shenzhen, China) for 1 h. After cooking, it was allowed to cool to room temperature and subsequently strained through a filter paper to remove any residue. The filtered broth was then stored in the refrigerator at −20 °C until further use.

2.9. Detection of AGEs content in pork soup

The AGEs level in the broth was detected using an AGE Assay Kit following the provided instructions. 150 μL sample or AGE standard solution were added into the AGEs conjugate coated plates, incubated on a shaker for 10 min at room temperature. Then 50 μL diluted Anti-AGEs Antibody was added into each well and incubated on a shaker for another 60 min. Following that, the wells were washed three times with 250 μL washing buffer added with 100 μL diluted secondary antibody-HRP conjugate, and shook for 60 min at room temperature. Washed three times with 250 μL washing buffer again. Then 100 μL substrate solution was added to each well and incubated on a shaker for 2 min. Finally, 100 μL stop solution was added to each well to stop the enzyme reaction. The absorbance of each well was measured by the multimode microplate reader at 450 nm.

2.10. Antiglycative activity-guided fractionation in black soybean sprouts

Black soybeans were sprouted and harvested on the 7th day. A total of 45 g sprouts were extracted using the method described in section 2.2. The extracted solution was then vacuum evaporated to dryness. Subsequently, an open column chromatography was employed using silica gel (100–200 mesh) or Sephadex LH20. For the silica gel separation, the elution buffer consisted of 500 mL ethyl acetate, 500 mL mixture of dichloromethane and methanol (1:1), 500 mL mixture of dichloromethane and methanol (1:2), 1000 mL dichloromethane and methanol (1:4), and 500 mL methanol. After separation, the organic solvent was evaporated to dryness using a rotary evaporator under reduced pressure at a 40 °C water bath. The resulting residue was re-dissolved in 10 mL methanol and then their AGEs inhibition rate was determined using the method mentioned in section 2.3. The fraction that exhibited the highest AGEs inhibition rate was selected and loaded onto a Sephadex LH20 column, with 500 mL methanol and 500 mL acetone as elution solvents (Q. Zhou et al., 2021). The fractions were from this step were collected separately and their AGEs inhibition ability was evaluated. The fraction that demonstrated the most promising antiglycative effects was chosen for further instrumental analysis.

2.11. Instrumental analysis of active components in black soybean sprouts

To analyze the active components presented in the final fraction, HPLC/UV and LC/MS techniques were employed. Briefly, 1 mL sample was added with 10 μL of 70% cold perchloric acid, centrifuged at 15000×*g* for 15 min, filtered through 0.22 μm membrane, and injected into the Waters HPLC system mentioned in section 2.4 and then to the Shimadzu LC/MS system (2050, Shimadzu Corporation, Kyoto, Japan). The mobile phase was 0.1% formic acid and ACN. A gradient elution method was employed, ranging 10%~90% in 35 min with a flow rate of 1 mL/min. The injection volume was 10 μL in HPLC analysis and 5 μL in LC/MS analysis. The parameters in the LC/MS program were set as follows: column temperature 40 °C, mass scan range 100~1000 m/z, UV scan range 190~800 nm, desolvation temperature 450 °C, nebulizing gas flow 2.0 L/min, drying gas flow 5.0 L/min, heating gas flow 7.0 L/min.

2.12. Quantification of active components in the seeds and sprouts of black soybean

The concentrations of the identified compounds from section 2.11 were evaluated by using HPLC/UV or LC/MS. Briefly, the extracts of black soybeans prepared in section 2.7 were 5-fold diluted, followed by chromatographic analyses employing the method mentioned in section 2.11. Standard curves were created for each compound by preparing a series of concentrations of the standard compound (0, 100, 200, 300, and 400 μM). The levels of active compounds were calculated by the following formula and the unit was nmol/bean or nmol/sprout.

$$\text{Active compound content} = \frac{x \times \text{diluted fold} \times \text{extract volume}}{\text{number of beans or sprouts}} = \frac{5x}{3}$$

x: the concentration of active compound in the diluted extract, μM; diluted fold = 5; extract volume = 10; and number of beans/sprouts = 30.

2.13. Statistical analysis

The data were presented as mean ± standard derivatization (SD) and were obtained from a minimum of three replicates. Each replication encompassed independent trials under controlled conditions to confirm the accuracy of the results. To compare the means of different groups, one-way analysis of variance (ANOVA) was performed and Tukey's test was adopted for multiple comparisons of all pairs. Statistical significance was defined as $p < 0.05$. GraphPad Prism 9.0 (GraphPad Software Inc., San Jose, CA, USA) was used for data analysis and figure generation, except for creating the Radar chart in sensory evaluation which were carried out by Microsoft Excel (2019) (Microsoft Corporation, Redmond, WA, USA).

3. Results and discussion

3.1. Enhanced antiglycative potential of beans through sprouting with black soybean exhibits the highest efficacy

The ripening cycle of bean sprouts was set as 7 days, according to the preliminary data and also with reference to literature data (Paśko et al., 2009). In the beginning, the length and weight changes of bean sprouts were recorded to know their primary growing characteristics. As shown in Fig. 1A–B, black soybean sprouted significantly taller than the remaining four beans. In particular, at the mature day (7th day), the length of black soybean, mung bean, pea, cowpea, and soybean were 18.92 ± 6.27 cm, 15.54 ± 2.97, 11.52 ± 2.31 cm, 12.88 ± 3.91 cm, and 14.02 ± 1.52 cm, respectively. As for the weight change, mung bean grew at a significantly slower rate than other beans. The final mature weight was 0.32 ± 0.09 g for mung bean sprout, but was at the range of 0.64–0.80 g for the other bean sprout. Following that, the antiglycative effect of the 7-day bean sprouts and their corresponded beans were evaluated in a fructose-BSA model, which mimicked the protein glycation response under hyperglycemic conditions (Jung, Park, Min, B, Jung, H. J., Islam, M. N., & Choi, 2015). Our experimental data showed that the inhibitory rate against AGEs formation by bean extracts was ranked as black soybean (12.92 ± 0.59%) ≈ soybean (11.44 ± 0.51%) ≈ pea (9.42 ± 1.25) > cowpea (4.40 ± 0.62%) ≈ mung bean (3.47 ± 0.48%). And the inhibition rate against AGEs formation by bean sprout extract was as follows: black soybean (69.40 ± 0.77%) > mung bean (49.51 ± 1.56%) ≈ cowpea (48.69 ± 2.66%) > pea (41.90 ± 2.01%) > soybean (8.59 ± 2.74%) (Fig. 1C). It is obvious that sprouting significantly enhanced the antiglycative activity of black soybean (by ~ 4.92-fold), mung bean, pea, and cowpea, although with no effect on soybean. Similarly, Liyanage et al. found that sprouted mung bean significantly reduced the blood glucose concentration of mice within high α-amylase and α-glucosidase inhibitory activity (Liyanage et al., 2018). Sprouted cowpea produced peptides to inhibit dipeptidyl peptidase IV (DPP-IV) activity, which can regulate the glucose level by the degradation of an incretin (i.e., glucagon-like peptide-1) (De Souza Rocha et al., 2014). These results confirmed that changes caused by the sprouting process of beans contribute to their enhanced activities. However, studies on the antiglycative properties of black soybean sprouts are limited. The present study had unveiled the heightened antiglycative potential of sprouted black soybean, prompting further investigations into the reasons and mechanisms responsible for their high efficiency.

The antiglycative capability of plants might be achieved via antioxidant, metal chelation, and trapping of reactive di-carbonyl species

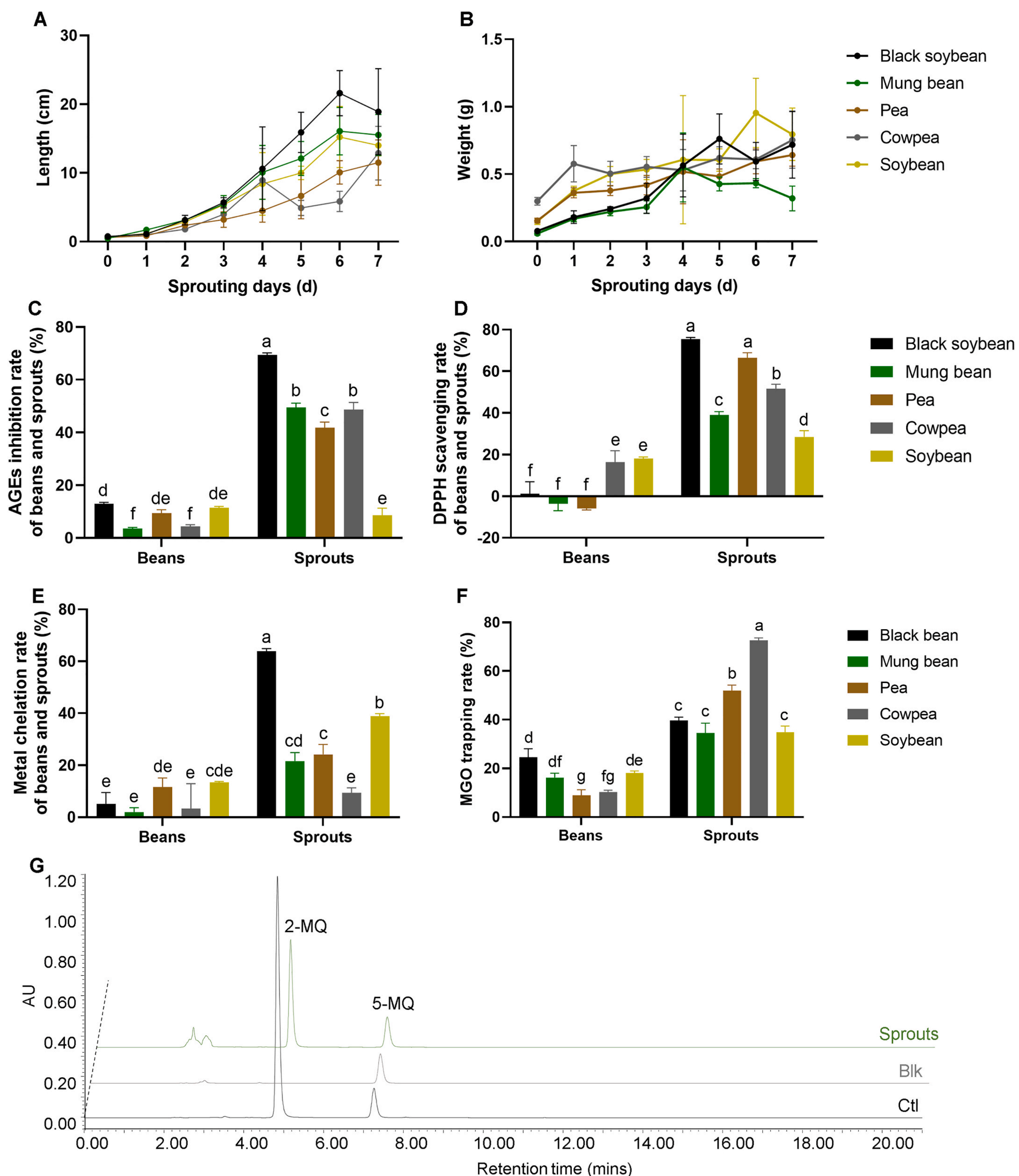

Fig. 1. Antiglycative analyses of beans and bean sprouts. A: Length change during sprouting process. B: Weight change during sprouting process. C: The AGEs inhibition rate in fructose-BSA model. D: DPPH scavenging activity. E: Metal chelation rate. F: MGO-trapping activity. G: HPLC chromatography of derivatized MGO and its internal standard. Columns with different characters are significantly different ($p < 0.05$).

(RCS) (Gao et al., 2020). We further evaluated whether bean and bean sprout extracts showed antiglycative effect through these three mechanisms. The antioxidant activity was detected by employing DPPH scavenging assay and our results showed that sprouting facilitated beans antioxidant activity significantly. Our experimental data showed that cowpea (16.34 ± 5.54%) and soybean (18.06 ± 0.82%) extracts have higher DPPH scavenging rate, while black soybean, mung bean, and pea showed little effect. Moreover, DPPH scavenging rate by bean sprout extract was as follows: black soybean (75.42 ± 0.86%) > pea (66.44 ± 2.55%) > cowpea (51.61 ± 2.19%) > mung bean (38.93 ± 1.74%) > soybean (28.55 ± 3.02%) (Fig. 1D). Notably, black soybean had the highest antioxidant potential (75.42 ± 0.86%), whilst the value was ~5.37 fold higher than the unsprouted black soybeans (1.21 ± 5.77%). In the metal chelating assay, sprouting also shows a significant enhancing effect on the chelating ability of black beans, mung beans, peas, and soybeans, among which black beans have the strongest chelating ability (Fig. 1E). Specifically, the metal chelation rate of beans was all lower than 20%. However, sprouting of black bean (63.91 ± 1.02%), soybean (38.80 ± 1.04%), pea (24.10 ± 3.90%), mung bean (21.61 ± 3.24%) significantly increase their metal chelation activity, with sprouted cowpea (9.38 ± 1.89%) showed no change to its bean (3.23 ± 9.68%). MGO was chosen as the representative RCS to show the trapping effect of beans and bean sprouts via HPLC analysis (Fig. 1F–G). Again, the MGO trapping potential of these beans were significantly increased after sprouting. Collectively, our results indicated that sprouting can increase the antiglycative activities of beans by the promoting of their antioxidant, metal chelating, and RCS trapping potentials, with black soybean showed the highest capability. Our data is in line with Miyahira et al., who summarized that sprouts improved the nutritional value and health benefits of food products (Miyahira et al., 2021). Additionally, we found current literature data were mainly focused on mung bean in the study of bean sprouts, where (1) approaches were proposed to facilitate the sprouting effect of mung bean, including chemical (e.g., sucrose and spermidine) and physical (e.g., plasma activated water and ultrasonics) treatment (Fan et al., 2020; Lyu et al., 2022; Millan-Sango et al., 2017; T. Zhou et al., 2020); (2) microbial quality and dynamics shift of harvested mung bean sprouts during shelf life (Iacumin and Comi, 2019; Keshri et al., 2019). However, our data outlined an elevated antiglycative and antioxidative potential of sprouts from black soybean than mung bean and other beans. This implied a prospective insight for the future study of black soybean sprouts.

3.2. *Black soybean sprouts as an effective antiglycative ingredient in food model*

Bean sprouts are commonly consumed fresh, stir-fried, or boiled in meat soup. However, the thermal processing of meat products can result in the formation of toxic compounds that induce oxidative stress and chronic inflammation, ultimately leading to a sub-healthy or pathological condition in human body. AGEs are one typical type of such toxicants found in meat (S. Huang et al., 2023). In light of this, we performed an assay to investigate the potential of black soybean in preventing the formation of AGEs in meat soup. Bean sprout and meat soup is a commonly consumed dish in the Lingnan region of China for a long time, owing to its stewing qualities and robust meaty flavor. An Abcam AGEs assay kit was employed to detect the level of AGEs in the soup. Deionized water was boiled as the control group (Ctl). LM, MM, and HM represented 50 g meat boiled with 15 g (LM), 30 g (MM), and 60 g (HM) sprout, respectively. Our data exhibited that the AGEs content decreased with the increment of black soybean sprouts in a dose-dependent manner (Fig. 2A). Additionally, the metal chelation rate in the meat-sprout soup was investigated, where HM (64.00 ± 0.29%) ≈ MM (62.06 ± 6.10%) > LM (50.87 ± 1.12%) > meat (11.97% ± 1.13) (Fig. 2B). This suggested the metal chelation ability of black soybean sprouts in the meat soup model. Intriguingly, the DPPH scavenging activity was also detected, but no significance can be calculated within the pure meat soup and meat-spout soups (Fig. 2C). This was unexpected. Therefore, we measured the antioxidant potential of soups from pure sprouts and pure meat by adopting the same processing method. As shown in Fig. 2D–E, both sprouts and meat had the potential to scavenge DPPH. As supported by Gil et al., the water in which pork was boiled for 1 h had high antioxidative effect, probably owing to the existence of bioactive peptides and proteins (Gil et al., 2016; Liu et al., 2016) This might be the reason why the adding level of spouts did not influence the DPPH scavenging rate of the sprout-meat soup. Pork is commonly used meat in most countries over the world, and its high fat content may increase the formation of AGEs in human body (Li et al., 2005). The addition of sprouted black soybeans significantly reduces the levels of AGEs, making this dish more edible for consumers at risk of

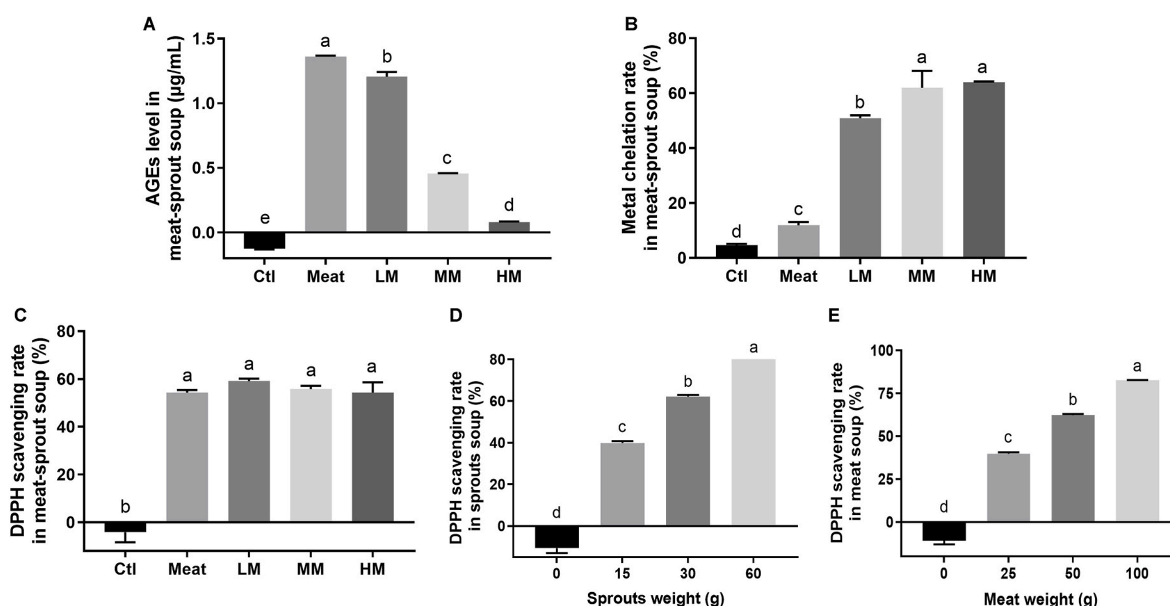

Fig. 2. Black soybean prevents AGEs formation in real food model. A: AGEs level in meat-sprout soups. B: Metal chelation rate in meat-sprout soup. C–E: DPPH scavenging rate of meat-sprout soup, pure sprout soup, and pure meat soup. Columns with different characters are significantly different ($p < 0.05$).

diabetes-related chronic diseases, as well as the elderly. Collectively, our data implied the beneficial effects of adding black soybean sprout in meat products. And to the best of our knowledge, few published data has addressed the antiglycative effect of sprouts in meat processing thus far.

3.3. Fractionation and characterization of the potential components in black soybean sprouts

To identify the active components, the matured black soybean sprouts were extracted and fractionated by our previous built anti-glycative activity-guided screening platform (Q. Zhou et al., 2023). In brief, six crude fractions were obtained, with SG-3 and SG-4 fraction showed significantly higher anti-glycation effects (Fig. 3A). Then SG-3 and SG-4 were combined and went through Sephadex LH20 open column for further separations. The results revealed that SL-6 fraction had the highest glycation inhibition rate and was chosen for chromatographic analysis (Fig. 3B). Our HPLC data exhibited 5 obvious peaks with a characteristic UV absorption spectrum of isoflavonoids (Fig. 3C–G). After that, LC/UV/MS analysis was performed. The TIC and typical XICs of SL-6 fraction were presented in Fig. 4A–F in positive mode. By comparing the UV absorption spectrum of HPLC and LC/MS, we identified the $[M+H]^+$ of these unknown compounds which were 417 (compound 1, RT = 11.31 min), 433 (compound 2, RT = 12.73 min), 255 (compound 4, RT = 15.06 min), 271 (compound 5, RT = 16.86 min), and 943 (compound 6, RT = 18.26 min), respectively. Unfortunately, compound 3 (in Fig. 3C) was not identified, because we cannot get the exact $[M+H]^+$ information. With reference to published data (Gu et al., 2017; G. Huang et al., 2017), these compounds were identified as daidzin, genistin, daidzein, genistein, and soyasaponin Bb, respectively (Fig. 4G–L). Our data are in consistent with Huang et al., who identified daidzein and genistein as the primary aglycones in germinated black soybean, with daidzin and genistin as the major β-glucosides (G. Huang et al., 2017). Similarly, Gu et al. reported that soyasaponin Bb is the predominant soyasaponin in soybean sprouts (Gu et al., 2017).

3.4. Isoflavones play a key role in the antiglycation of black soybean sprouts

After identifying the potential antiglycative contributors in black soybean, we evaluated impact of sprouting on the health benefits (i.e., antiglycative and antioxidant abilities) and bioactive compounds (i.e., total phenols, isoflavones, and soyasaponin Bb). As elucidated in Fig. 5A, the AGEs inhibition rate in the sprouts raised significantly from

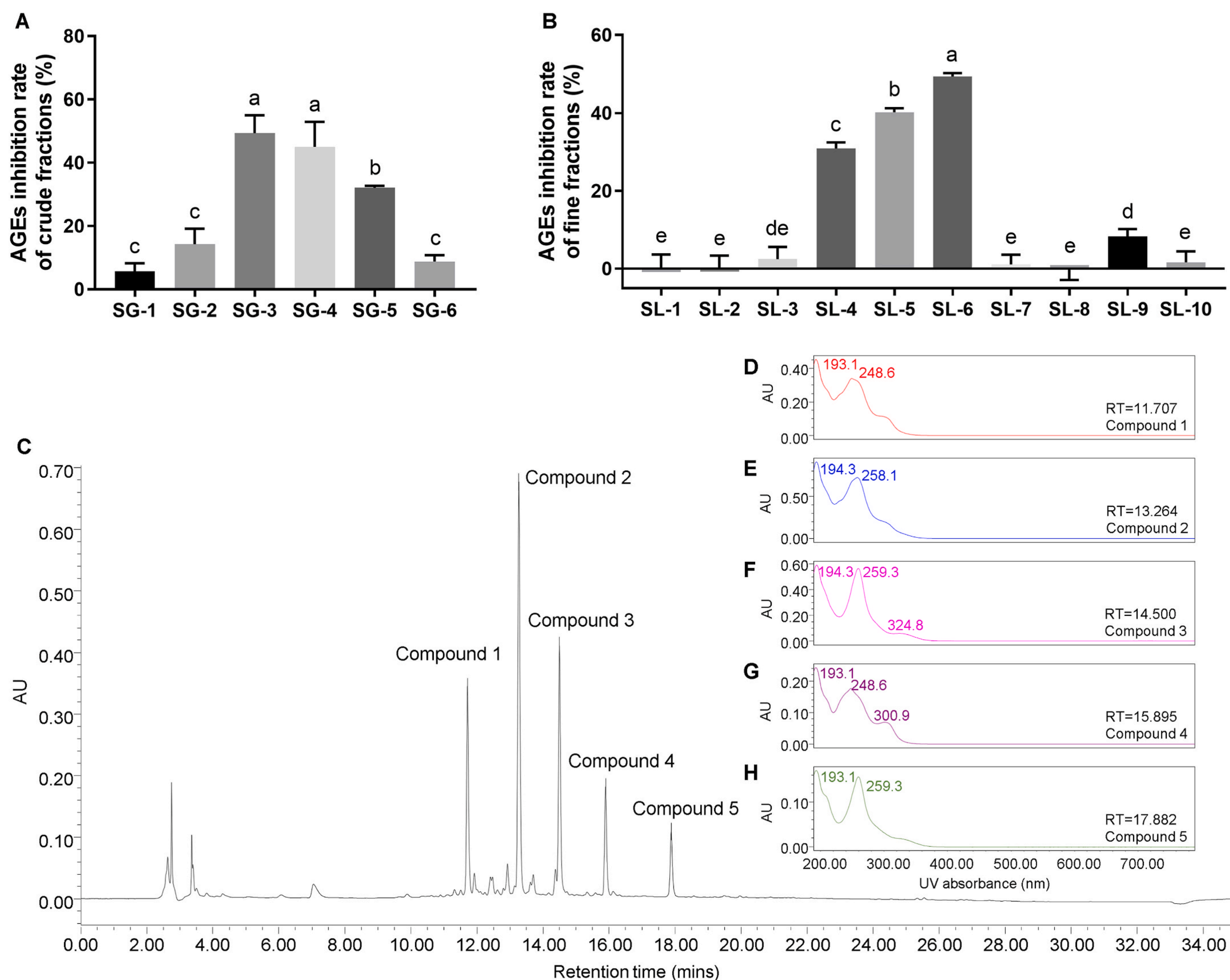

Fig. 3. Characterization of active components in black soybean sprouts. A: AGEs inhibition rate of eluents from silica gel column fractionation. B: AGEs inhibition rate of eluents from Sephadex LH20 column fractionation. C. HPLC chromatogram of SL-6 fraction. D–H: UV absorbances of compound 1–5. Columns with different characters are significantly different ($p < 0.05$).

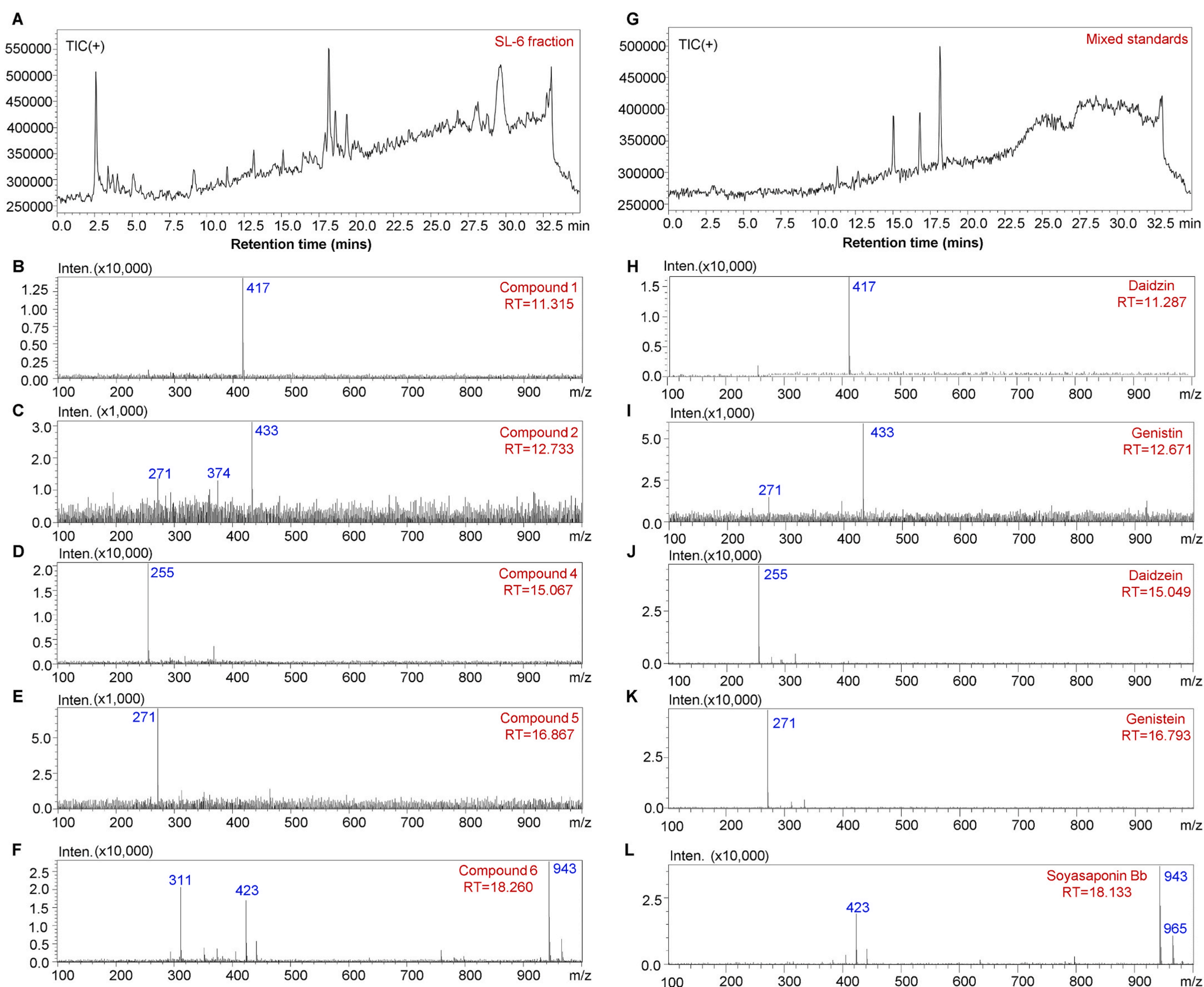

Fig. 4. LC/MS results of SL-6 fraction and identified standards. A: TIC of SL-6 fraction in positive mode. B–F: XICs of compound 1–2 and 4–6. G: TIC of mixed standards. H–L: XICs of daidzin, genistin, daidzein, genistein, and soyasaponin Bb.

the 3rd day (18.30 ± 0.76%) and then increased linearly to 70.09 ± 0.78% at the 7th day. The *in vitro* antioxidant activity and total phenolic content shared a similar trend in black soybean sprouts, which increased significantly from the 1st day of sprouting (Fig. 5B–C). The DPPH scavenging rate at the 7th day was 2.51-fold higher than the ungerminated beans and was 1.20-fold higher than at the 1st day. In the comparison of total phenolic content, a standard curve was constructed as y = 2.3966x + 0.9909, where y represented the concentration of the standard solution and x represented $OD_{760\ nm}$. The coefficient of determination (R^2) for the standard curve was 0.9909. The concentration range was 0.00488–0.625 mg/mL. The results were 7.28- and 2.51-fold higher in 7th day versus ungerminated and 7th day versus 1st day. Fig. 5D presented the HPLC chromatography of the black soybean sprouts, beans, and the isoflavone standards. And sprouting facilitated the accumulation of isoflavones and soyasaponin Bb (Fig. 5E). Our findings was in consistent with previous report that also showed the accumulation of isoflavones after sprouting of beans (X. Huang et al., 2014). Notably, the unit nmol/sprout was adopted so as to compare the molar changes within these compounds during the sprouting process. With reference to their maximum UV absorbance (Fig. 3), daidzin and daizein were quantified at the wavelength of 254 nm, genistin and genistein at 258 nm. Soyasaponin Bb was detected at 190 nm. The equation of the standard curves for daidzin, genistin, daidzein, genistein, and soyasaponin Bb were as follows: y = 9310.1x + 6249.8 (R^2 = 0.9997), y = 12028x – 72608 (R^2 = 0.9826), y = 8757.8x + 13998 (R^2 = 0.9881), y = 10720x + 11056 (R^2 = 0.9997), and y = 2148.3x - 8106.6 (R^2 = 0.9990). The concentration range for all standards were 0~400 μM. The LOD and LOQ of standards were 0.66 nM and 1.99 nM for daidzin, 346.62 nM and 1039.86 nM for genistin, 62.77 nM and 188.30 nM for daidzein, 1.02 nM and 3.06 nM for genistein, 12.45 nM and 37.36 nM for soyasaponin, respectively. Generally, the seeds of black soybean contained glycosides daidzin (21.42 ± 0.20 nmol/bean) and genistin (157.01 ± 0.13 nmol/bean), whereas their aglycones daidzein and genistein were below detection. During sprouting, daidzin and genistin rapidly increased on day 1 (95.42 ± 0.31 and 242.06 ± 0.40 nmol/sprout) and showed moderate increments until day 7 (203.15 ± 0.35 and 318.49 ± 0.46 nmol/sprout). On the contrary, daidzein and genistein gradually increased from day 1 (4.82 ± 0.02 and 3.88 ± 0.20 nmol/sprout) to day 6 (18.03 ± 0.31 and 9.21 ± 0.20 nmol/sprout) and then sharply raised to 73.55 ± 0.68 and 53.81 ± 1.59 nmol/sprout at day 7. This implied that the sprouts contained higher amounts of flavonoid glycosides compared to their aglycones. Additionally,

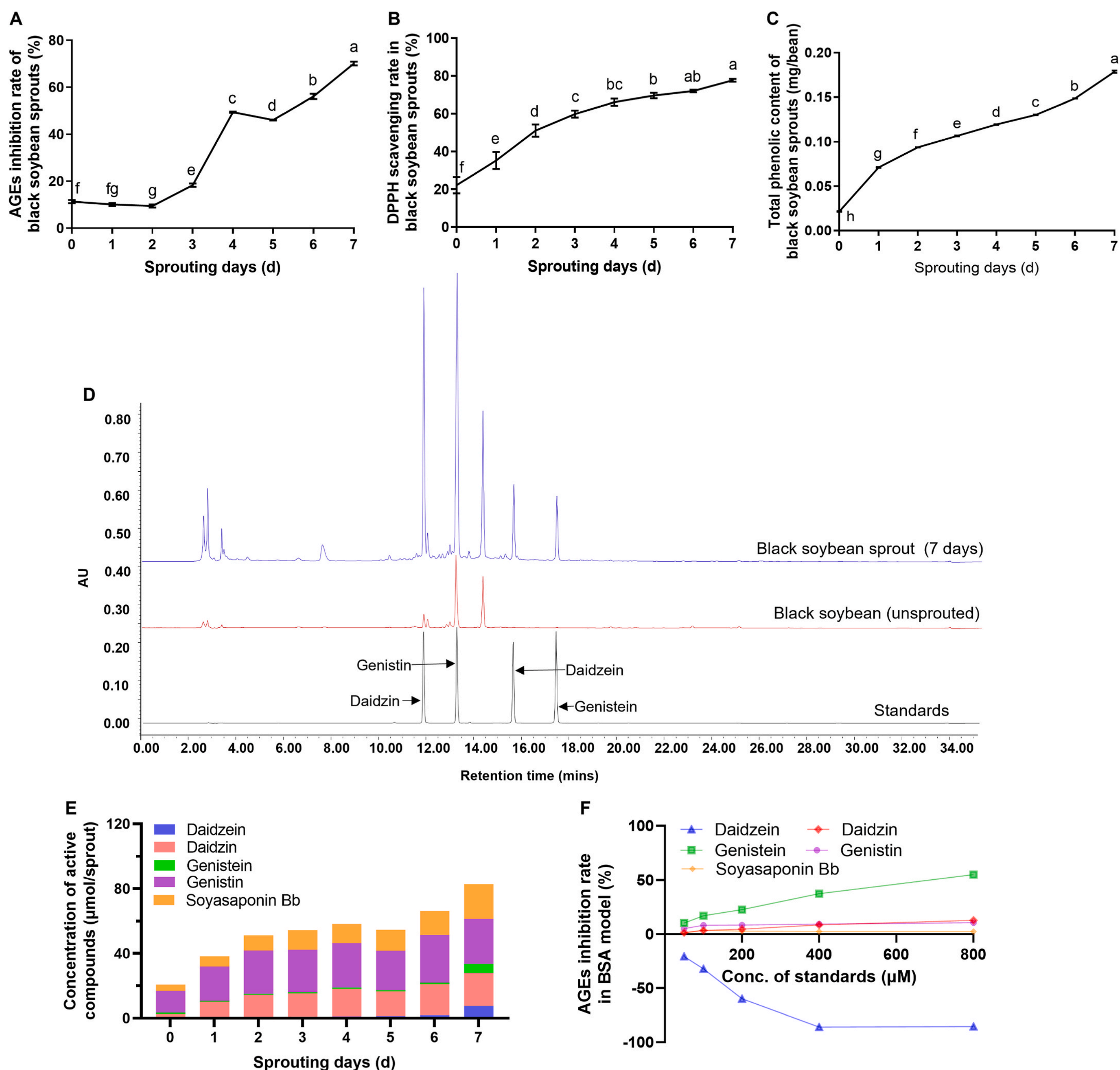

Fig. 5. The sprouting benefits of black soybeans. A: AGEs inhibition rate during sprouting. B: DPPH scavenging rate during sprouting. C: Total phenolic content during sprouting. D: HPLC chromatograms of sprouts, beans, and standards. E: Isoflavones and soyasaponin Bb concentrations during sprouting. F: AGEs inhibition rate of standards.

glycosides tent to be accumulated in the early stages, while aglycones were formed at later stages of sprout processing. After that, the AGEs inhibition rate of the potential compounds was measured in the BSA model (Fig. 5F). The interesting thing is that genistein possessed a dose-dependent effect on preventing AGEs formation peaked at 54.98 ± 0.19%, while genistin had the highest rate (10.51 ± 1.49%) at the concentration of 800 μM. This is in line with our previous findings which mentioned the decreasing of antiglycative activity of glycosides than aglycones (Q. Zhou et al., 2020). Meanwhile, daidzin showed an antiglycative rate of 12.80 ± 0.91% at 800 μM, whereas daidzein exerted no appreciable effects on AGEs. Our results were supported by Genova et al., where daidzein had a maximum AGEs inhibition rate of 2.33% at 950 μg/mL (3.8 mM) (Genova et al., 2021). Soyasaponin Bb also showed no effect on AGEs formation. Collectively, it can be concluded that the antiglycative effect of black soybean sprouts can be primarily ascribed to the accumulation of genistein, genistin, and daidzin. Thus, our findings indicate that sprouting is a practical strategy to promote the antiglycative and antioxidant effects of black soybean, as well as to increase the formation of isoflavones.

4. Conclusions

In the present study, we investigated the sprouting benefits of five common beans from the perspective of antiglycation in a model system. Our data indicated that sprouting can enhance the antiglycative effect of black soybean, mung bean, pea, and cowpea, with black soybean

exhibiting the highest potential. Sprouting also promoted the *in vitro* antioxidant, metal chelation, and RCS-scavenging activity of beans, contributing to their antiglycative effect. Following that, black soybean sprouts were further proved to prevent AGEs formation in pork-sprout soup, which is a food model that sprouts are commonly consumed. The active components were characterized as isoflavones (daidzin, genistin, daidzein, and genistein) via open column fractionation. Moreover, during the sprouting process of black soybean, its antiglycation, antioxidation, total phenolics, and isoflavones increased significantly. However, genistein, genistin, and daidzin were identified as the major contributors in black soybean sprouts by the comparison of their concentration and antiglycative activity. Collectively, this study demonstrated the sprouting benefits of beans and implied isoflavones as the primary antiglycative components in black soybean. These findings highlighted the potential of bean sprouts, particularly black soybean sprouts, as functional foods with notable antiglycative attributes. This insight may foster innovations in food science and inspire the creation of sprouting-based dietary strategies to combat glycation-related chronic diseases and slow the aging process, thereby improving public health and promoting healthier eating habits.

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CRediT authorship contribution statement

Qian Zhou: Conceptualization, Methodology, Writing – original draft, Writing – review & editing, Visualization, Funding acquisition. **Yuxuan Chen:** Methodology, Writing – original draft, Validation. **Lifang Peng:** Methodology, Writing – original draft, Validation. **Jun Wu:** Methodology, Formal analysis, Writing – review & editing. **Wen Hao:** Formal analysis, Writing – review & editing. **Mingfu Wang:** Investigation, Writing – review & editing, Supervision, Project administration, Funding acquisition.

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.

Data availability

Data will be made available on request.

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