Fermented Soybean-Derived Cheonggukjang Physiological Activities

Subjects: Food Science & Technology
Contributor: Il-Sup Kim

Cheonggukjang (CGJ, fermented soybean paste), a traditional Korean fermented dish, has recently emerged as a functional food that improves blood circulation and intestinal regulation. Considering that excessive consumption of refined salt is associated with increased incidence of gastric cancer, high blood pressure, and stroke in Koreans, consuming CGJ may be desirable, as it can be made without salt, unlike other pastes. Soybeans in CGJ are fermented by Bacillus strains (B. subtilis or B. licheniformis), Lactobacillus spp., Leuconostoc spp., and Enterococcus faecium, which weaken the activity of putrefactive bacteria in the intestines, act as antibacterial agents against pathogens, and facilitate the excretion of harmful substances.

Keywords: fermented soybean paste ; cheonggukjang ; bioactive molecule ; biological activity ; human health benefit

1. Introduction

Cheonggukjang (CGJ) is a traditional Korean dish produced by fermenting boiled soybeans rice straw, which naturally contains *Bacillus subtilis*. Fresh CGJ is prepared by spreading rice straw on boiled soybeans and keeping them warm at 40–50 °C for 2–3 days (Figure 1A) [1][2][3][4][5]. During the fermentation process of CGJ, soy protein is decomposed into amino acids by potent proteolytic enzymes produced by *B. subtilis* (Figure 1B), thus improving digestibility and increasing the vitamin B_2 and calcium contents of the final product [6]. The sticky mucous substance produced during fermentation contains poly-γ-glutamic acid (γ-PGA), the main component, and altered isoflavone compounds. Polyglutamic acid is beneficial to health, as it aids in the absorption of calcium [7][8]. Isoflavone, the main physiologically active substance in soybeans, improves the absorption and bioavailability of nutrients from the fermented soybeans [2][9][10].

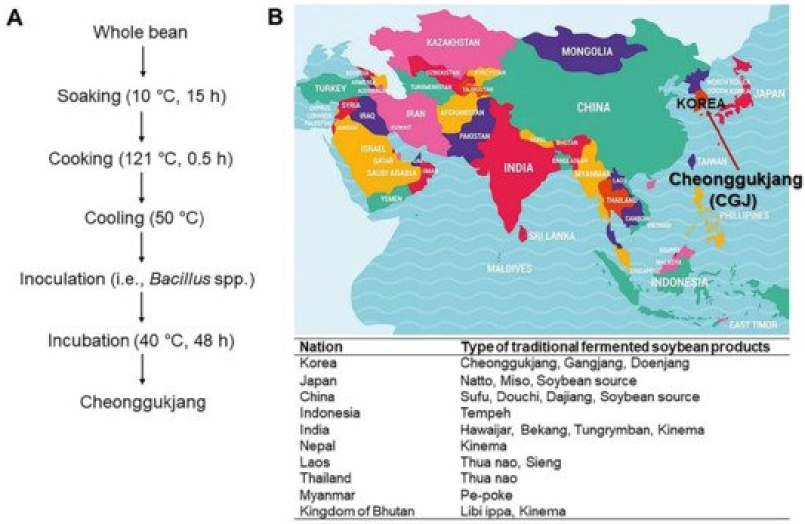

Figure 1. General procedure for preparation of CGJ (**A**) [2] and the broad type of traditional fermented soybean products in Asia (**B**) [5].

Rich in microorganisms, enzymes, and various physiologically active substances, CGJ has gained attention as a functional food to help maintain and promote health by improving intestinal motility and blood circulation [4][5]. Currently, raw and powdered forms of CGJ are being developed. Unlike natto, which is a traditional Japanese dish consisting of fermented soybeans, and other soybean-fermented pastes in Asia that are eaten raw, CGJ does not contain salt, but can include ingredients such as crushed green onions, garlic, and red pepper powder for flavor, and salt can be added to the boiled soybeans to extend the product's shelf life [3][4][5]. This provides an advantage over kimchi and other soybean paste products that contain high salt concentrations, a factor that is associated with gastric cancer and high blood pressure [11]

[12]. Moreover, CGJ can be produced within 2–3 days, whereas doenjang, another soybean paste, takes several months to manufacture [2][10][13]. CGJ, with its unique flavor, is recognized as the most nutritionally and economically effective way to consume soybeans.

Studies on CGJ have mainly focused on the fermentation process and biological activities of CGJ. Consumption of soy-based foods has been shown to prevent cancer, particularly reducing the incidence of breast and prostate cancers [14]. CGJ contains large amounts of various physiologically active substances, including γ-PGA, isoflavones, phytic acid, saponins, trypsin inhibitors, tocopherols, unsaturated fatty acids (i.e., conjugated linoleic acid (CLA)), dietary fiber, oligosaccharides, itutin A, bacillomycin D, pharmaceutical and industrial enzymes (including protease, amylase, and cellulase), and possesses antibacterial activity (Table 1) [2][4][5][10]. CGJ is known to have thrombolytic effects owing to its protease activity. It also contains daidzein, an isoflavone (Figure 2), which has immunomodulatory effects that involve stimulation of estrogen receptor β (ER-β) [2][4][5]. Cell signaling mediated by ER-β is associated with regulation of antioxidant gene expression, the immune response, apoptosis, blood pressure, and inhibition of breast, prostate, and colon cancer cell proliferation [10][15][16]. In addition, oligosaccharides released from CGJ by the action of β-glucanases have various physiological activities, including diabetes prevention [10][17][18]. Free radicals generated in the body due to excessive drinking, smoking, overeating, and mental stress—which are linked to cancer, atherosclerosis, accelerated aging, and inflammation [19][20][21][22]—can be countered by antioxidants (such as amino acids, chlorogenic acid, caffeic acid, and browning substances) and isoflavones (such as genistein and daidzein) found in CGJ [2][4][5][23]. In addition, CGJ reportedly contains higher antioxidant levels than non-fermented soybeans [24]. CGJ also contains peptides that can lower blood pressure by acting as angiotensin I-converting enzyme (ACE) inhibitors [4][5][10][25]. ACE converts angiotensin I to angiotensin II, increasing blood pressure in vivo [26]. Thus, CGJ contains physiologically active substances that have anticancer, antioxidant, and blood pressure-lowering activities, as well as hypertension and osteoporosis prevention properties.

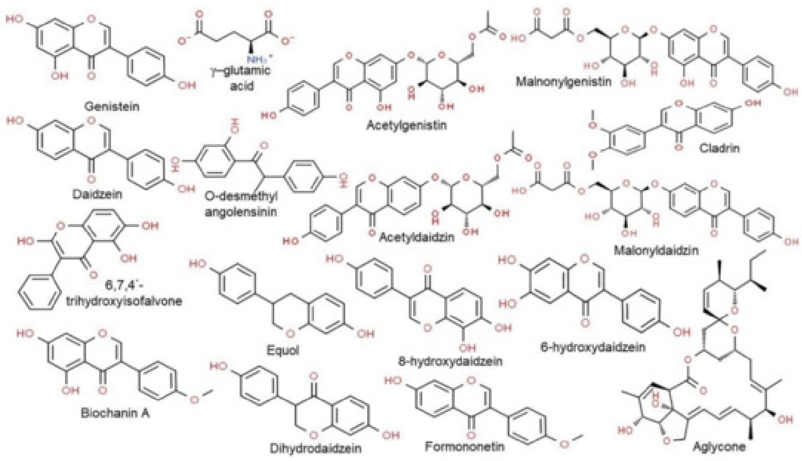

Figure 2. Chemical structure of the major classes of isoflavone and metabolites and γ-glutamic acid produced by CGJ fermentation. Structures were drawn using the ChemSpider (Royal Society of Chemistry, Cambridge, UK; http://www.chemspider.com, accessed on 24 May 2021) tool.

Table 1. The properties of CGJ fermented with different *Bacillus* species [3].

	B. amyloliquefaciens			*B. subtilis*	Soybeans
	SCGB1	SRCM 100730	SRCM 100731	SCGB 574	
γ-PGA (cm)	31 ± 0.86	27 ± 100	30 ± 0.57	55 ± 1.00	0
Flavor	D (++)	D (++)	D (++)	D (++)	ND
Protease activity (cm)	2.84 ± 0.04	1.94 ± 0.08	1.92 ± 0.12	2.29 ± 0.04	1.76 ± 0.01
Cellulase activity (cm)	2.08 ± 0.04	1.58 ± 0.04	1.44 ± 0.04	1.95 ± 0.07	1.78 ± 0.04
Amylase activity (cm)	2.84 ± 0.04	2.29 ± 0.05	2.42 ± 0.11	2.29 ± 0.04	1.99 ± 0.01
Thrombolytic activity (halo size, cm)	1.83 ± 0.06	3.85 ± 0.02	4.07 ± 0.14	1.95 ± 0.15	ND
Antibacterial activity	D (+++)	D (+++)	D (+++)	D (+++)	ND
Iturin A	D (+)	D (+++)	D (+++)	D (++)	ND

	B. amyloliquefaciens			*B. subtilis*	Soybeans
	SCGB1	SRCM 100730	SRCM 100731	SCGB 574	
Bacillomycin D	D (+++)	D (+++)	D (+++)	D (+++)	ND

The people of Asia have been producing special foods with unique flavors by adding different microbial strains (e.g., yeast or *Bacillus* spp.) to steamed soybeans for thousands of years [1][2][3][4]. The process of microbial fermentation not only imparts a unique flavor, but also enhances the nutritional value, shelf life, and bioactive substance content [10][27]. Research and development of food products is often based on the traditional foods of the in country in which the research is conducted. Asian countries, including Korea and Japan, have mainly investigated soybean-based fermented foods, whereas Europe and North America have actively conducted research on fermented foods such as cheese, wine, and beer [10][28]. The European Union organized a working group to provide extensive support to researchers investigating traditional foods to determine safety control measures, enhance their nutritional value, and promote marketing of these foods [29]. While continuous efforts have been directed toward researching fermented foods in Korea, a unified support system for such research has yet to be established at the national level [10][27][28]. At present, research is performed sporadically without an integrated database to converge study findings and there is a general lack of comprehensive discussion regarding the health functionality of traditional fermented foods [10][30]. In this review, we examine recent information on the novel physiological activities of CGJ, including anti-obesity, anti-diabetes, anti-inflammatory, and antimicrobial activities as well as immunostimulatory, neuroprotective, and skin improvement effects based on the following aspects: (1) nutritional properties of CGJ, (2) identification and characterization of microorganisms used in fermentation, and (3) health functionality assessment. Furthermore, this review aims to suggest future directions for study through the examination of CGJ characteristics, with a special focus on health functionality assessment.

2. Nutritional Properties of CGJ

CGJ is a well-known fermented food mainly produced between fall and early spring in Korea, as shown in Figure 1. Soybeans, the base ingredient of CGJ, comprises approximately 40% proteins and 20% lipids, resembling meat rather than grains in terms of nutritional values. Soybeans also contain 12% dietary fibers (2.3% soluble dietary fibers and 9.7% insoluble dietary fibers), which is not present in beef, and lacks cholesterol (Figure 3A) [1][2][10], which is associated with various diseases [31]. Aside from its unique flavor, CGJ ensures the most effective intake of soybeans, easily providing protein that may be deficient in Korean diets that are mainly composed of cereal grains [4]. Additionally, CGJ has higher protein and fat contents than doenjang (soybean paste) or gochujang (red chili paste) [24][31]. Hence, it has been an essential source of protein for a long time, along with other paste-based foods, for Koreans with a relatively low protein intake.

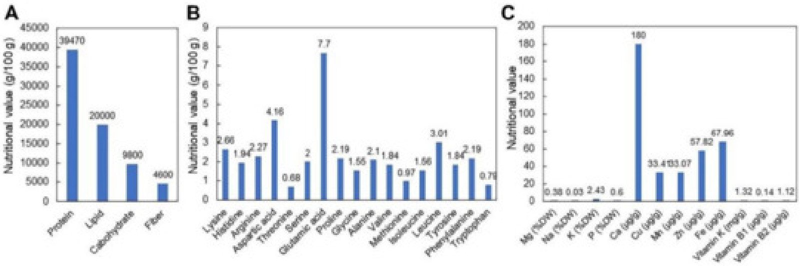

Figure 3. Proximate composition (**A**), concentration of amino acids (**B**) and mineral and vitamin content (**C**) of CGJ [1][2].

Different enzymes secreted by CGJ-producing bacteria during fermentation mediate degradation of the soybean outer coat, cell membrane fibers, and intracellular sugars and proteins to improve digestibility and increase the free amino acids contents [32]. During fermentation, proteins are degraded into peptone, polypeptides, dipeptides, and amino acids through protease activities (Figure 3B) [33]. β-amylase mediates the degradation of carbohydrates into glucose [34]. The sticky substance generated during CGJ fermentation is the combined product of the polysaccharide fructan and the amino acid polyglutamic acid [4][5][33]. There is a substantial increase in the vitamin B_2 level during fermentation; the vitamin B_2 content is 5- to 10-fold higher in steamed than in raw soybeans. Among the substances produced by CGJ bacteria, vitamin K (menaquinone; types K_1 and K_2) (Figure 3C) is required for the synthesis of proteins involved in blood coagulation [2][6][35][36]. In raw soybeans, vitamin K_1 is present in trace amounts and the vitamin K_2 level is negligible. However, CGJ contains low levels of vitamin K_1 but 5- to 10-fold higher levels of vitamin K_2 than other vegetables [1][2][3][4][10]. As vitamin K_2 is directly engaged in osteogenesis, vitamin K deficiency may increase the risk of fracture [6][24][31][35][36]. In a study in Japan, the use of *B. subtilis* for natto fermentation was reportedly the most effective method of improving vitamin K_2 intake [2][10][37]. Thus, the bioactive effects of CGJ can be predicted from the main microbial strain (*Bacillus*) used for fermentation.

References

1. Lee, B.Y.; Kim, D.N.; Kim, K.H. Physico-chemical properties of viscous substance extracted from chungkook-jang. Kore an J. Food Sci. Technol. 1991, 23, 599–604.
2. Lee, J.O.; Ha, S.D.; Kim, A.J.; Yuh, C.S.; Bang, I.S.; Park, S.H. Industrial application and physiological functions of cho ngkukjang. Food Sci. Ind. 2005, 38, 69–78.
3. Jeong, D.Y.; Ryu, M.S.; Yang, H.J.; Park, S. Gamma-PGA-rich chungkookjang, short-term fermented soybeans: Preven ts memory impairment by modulating brain insulin sensitivity, neuro-inflammation, and the gut-microbiome-brain axis. F oods 2021, 10, 221.
4. Jang, C.H.; Oh, J.; Lim, J.S.; Kim, H.J.; Kim, J.S. Fermented soy products: Beneficial potential in neurodegenerative di seases. Foods 2021, 10, 636.
5. Liu, L.; Chen, X.; Hao, L.; Zhang, G.; Jin, Z.; Li, C.; Yang, Y.; Rao, J.; Chen, B. Traditional fermented soybean products: Processing, flavor formation, nutritional and biological activities. Crit. Rev. Food Sci. Nutr. 2020, 1–19.
6. Tamang, J.P.; Shin, D.H.; Jung, S.J.; Chae, S.W. Functional properties of microorganisms in fermented foods. Front. Mi crobiol. 2016, 7, 578.
7. Marco, M.L.; Heeney, D.; Binda, S.; Cifelli, C.J.; Cotter, P.D.; Foligne, B.; Ganzle, M.; Kort, R.; Pasin, G.; Pihlanto, A.; et al. Health benefits of fermented foods: Microbiota and beyond. Curr. Opin. Biotechnol. 2017, 44, 94–102.
8. Sanlier, N.; Gokcen, B.B.; Sezgin, A.C. Health benefits of fermented foods. Crit. Rev. Food Sci. Nutr. 2019, 59, 506–52 7.
9. Hsiao, Y.H.; Ho, C.T.; Pan, M.H. Bioavailability and health benefits of major isoflavone aglycones and their metabolites. J. Funct. Foods 2020, 74, 104164.
10. Mun, E.G.; Kim, B.; Kim, E.Y.; Lee, H.J.; Kim, Y.; Park, Y.; Cha, Y.S. Research trend in traditional fermented foods focus ed on health functional evaluation. J. Korean Soc. Food Sci. Nutr. 2018, 47, 373–386.
11. Nan, H.M.; Park, J.W.; Song, Y.J.; Yun, H.Y.; Park, J.S.; Hyun, T.; Youn, S.J.; Kim, Y.D.; Kang, J.W.; Kim, H. Kimchi and soybean pastes are risk factors of gastric cancer. World J. Gastroenterol. 2005, 11, 3175–3181.
12. Park, S.; Kim, M.J.; Hong, J.; Kim, H.J.; Yi, S.H.; Lee, M.K. Selection and characterization of Cheonggukjang (fast ferm ented soybean paste)-originated bacterial strains with a high level of S-adenosyl-L-methionine production and probiotic s efficacy. J. Med. Food 2014, 17, 1170–1176.
13. Shin, D.; Jeong, D. Korean traditional fermented soybean products: Jang. J. Ethn. Foods 2015, 2, 2–7.
14. Takagi, A.; Kano, M.; Kaga, C. Possibility of breast cancer prevention: Use of soy isoflavones and fermented soy bever age produced using probiotics. Int. J. Mol. Sci. 2015, 16, 10907–10920.
15. Zhang, Z.; Zhou, L.; Xie, N.; Nice, E.C.; Zhang, T.; Cui, Y.; Huang, C. Overcoming cancer therapeutic bottleneck by dru g repurposing. Signal Transduct. Target. Ther. 2020, 5, 113.
16. Yan, X.; Qi, M.; Li, P.; Zhan, Y.; Shao, H. Apigenin in cancer therapy: Anti-cancer effects and mechanisms of action. Cel l Biosci. 2017, 7, 50.
17. Wirngo, F.E.; Lambert, M.N.; Jeppesen, P.B. The physiological effects of dandelion (Taraxacum officinale) in type 2 diab etes. Rev. Diabet. Stud. 2016, 13, 113–131.
18. Gilbert, E.R.; Liu, D. Anti-diabetic functions of soy isoflavone genistein: Mechanisms underlying its effects on pancreatic beta-cell function. Food Funct. 2013, 4, 200–212.
19. Papachristoforou, E.; Lambadiari, V.; Maratou, E.; Makrilakis, K. Association of glycemic indices (hyperglycemia, glucos e variability, and hypoglycemia) with oxidative stress and diabetic complications. J. Diabetes Res. 2020, 2020, 748979 5.
20. Schreihofer, D.A.; Oppong-Gyebi, A. Genistein: Mechanisms of action for a pleiotropic neuroprotective agent in stroke. Nutr. Neurosci. 2019, 22, 375–391.
21. Sies, H. Oxidative stress: A concept in redox biology and medicine. Redox Biol. 2015, 4, 180–183.
22. Pisoschi, A.M.; Pop, A. The role of antioxidants in the chemistry of oxidative stress: A review. Eur. J. Med. Chem. 2015, 97, 55–74.

23. Zhu, Y.L.; Zhang, H.S.; Zhao, X.S.; Xue, H.H.; Xue, J.; Sun, Y.H. Composition, distribution, and antioxidant activity of phenolic compounds in 18 soybean cultivars. J. AOAC Int. 2018, 101, 520–528.

24. Ali, M.W.; Shahzad, R.; Bilal, S.; Adhikari, B.; Kim, I.D.; Lee, J.D.; Lee, I.J.; Kim, B.O.; Shin, D.H. Comparison of antioxidants potential, metabolites, and nutritional profiles of Korean fermented soybean (Cheonggukjang) with Bacillus subtilis KCTC 13241. J. Food Sci. Technol. 2018, 55, 2871–2880.

25. Mallikarjun Gouda, K.G.; Gowda, L.R.; Rao, A.G.; Prakash, V. Angiotensin I-converting enzyme inhibitory peptide derived from glycinin, the 11S globulin of soybean (Glycine max). J. Agric. Food Chem. 2006, 54, 4568–4573.

26. Serfozo, P.; Wysocki, J.; Gulua, G.; Schulze, A.; Ye, M.; Liu, P.; Jin, J.; Bader, M.; Myohanen, T.; Garcia-Horsman, J.A.; et al. Ang II (angiotensin II) conversion to angiotensin-(1-7) in the circulation is POP (prolyloligopeptidase)-dependent and ACE2 (angiotensin-converting enzyme 2)-independent. Hypertension 2020, 75, 173–182.

27. Mukherjee, R.; Chakraborty, R.; Dutta, A. Role of fermentation in improving nutritional quality of soybean meal—A review. Asian-Australas. J. Anim. Sci. 2016, 29, 1523–1529.

28. Ashaolu, T.J.; Reale, A. A holistic review on Euro-Asian lactic acid bacteria fermented cereals and vegetables. Microorganisms 2020, 8, 1176.

29. Magnuson, B.; Munro, I.; Abbot, P.; Baldwin, N.; Lopez-Garcia, R.; Ly, K.; McGirr, L.; Roberts, A.; Socolovsky, S. Review of the regulation and safety assessment of food substances in various countries and jurisdictions. Food Addit. Contam. Part A Chem. Anal. Control Expo. Risk Assess 2013, 30, 1147–1220.

30. Xiang, H.; Sun-Waterhouse, D.; Waterhouse, G.I.N.; Cui, C.; Ruan, Z. Fermentation-enabled wellness foods: A fresh perspective. Food Sci. Hum. Well. 2019, 8, 203–243.

31. Shahzad, R.; Shehzad, A.; Bilal, S.; Lee, I.J. Bacillus amyloliquefaciens RWL-1 as a new potential strain for augmenting biochemical and nutritional composition of fermented soybean. Molecules 2020, 25, 2346.

32. Sharma, R.; Garg, P.; Kumar, P.; Bhatia, S.K.; Kulshrestha, S. Microbial fermentation and its role in quality improvement of fermented foods. Fermentation 2020, 6, 106.

33. Chatterjee, C.; Gleddie, S.; Xiao, C.W. Soybean bioactive peptides and their functional properties. Nutrients 2018, 10, 1211.

34. Ceusters, N.; Frans, M.; Van den Ende, W.; Ceusters, J. Maltose processing and not beta-amylase activity curtails hydrolytic starch degradation in the CAM orchid phalaenopsis. Front. Plant Sci. 2019, 10, 1386.

35. Wu, W.J.; Lee, H.Y.; Lee, G.H.; Chae, H.J.; Ahn, B.Y. The antiosteoporotic effects of Cheonggukjang containing vitamin K2 (menaquinone-7) in ovariectomized rats. J. Med. Food 2014, 17, 1298–1305.

36. Wu, W.J.; Gao, H.; Jin, J.S.; Ahn, B.Y. A comparatively study of menaquinone-7 isolated from Cheonggukjang with vitamin K1 and menaquinone-4 on osteoblastic cells differentiation and mineralization. Food Chem. Toxicol. 2019, 131, 110540.

37. Pradhananga, M. Effect of processing and soybean cultivar on natto quality using response surface methodology. Food Sci. Nutr. 2019, 7, 173–182.