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Review

Application of lactic acid bacteria-derived GABA in food industry: GABA-producing strains, biosynthesis, and health benefits

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Abstract

In recent years, there has been rising interest in producing health-functional foods and beverages as people become more health-conscious. Amidst the interest, gamma-aminobutyric acid (GABA), a key bioactive compound produced by lactic acid bacteria (LAB), is one of the most demanded compounds to be applied in the food industry due to its psychobiotic advantages. However, the limited number of studies focusing on specific areas of GABA application in the food industry complicates the general evaluation of its application. Hence, a systematic literature review was conducted to study the application of GABA in the food industry. Thirty-seven studies were chosen from PubMed, Web of Science (WoS), and Scopus for data extraction and review. The three themes being emphasised in the present review are (1) GABA-producing strains, (2) GABA biosynthesis, and (3) GABA health benefits. Through the present review, the general idea of how GABA is applied in the food industry is highlighted. This may provide a better overview of the current research direction on GABA.

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Introduction

Lactic acid bacteria (LAB) constitute a significant group of Gram-positive bacteria that produce a variety of primary metabolites and enzymes, including lactic acid, conjugated linoleic acid, vitamins, and bioactive compounds. They also have distinctive physiological activities (Li and Cao, 2010). LAB is not a new concept in the food industry; they have been widely used for centuries, and are considered safe for usage, as they can increase the shelf life of highly perishable raw matrices *via* lactic acid fermentation (Ağagündüz *et al*., 2022).

The U.S. Food and Drug Administration (FDA) designated LAB as GRAS or Generally Recognized As Safe, making them a prominent and well-known component used in dairy products, breads, fermented vegetables, meats, and fish (Li and Cao, 2010). Due to their lactic acid fermentative activity, LAB are a popular choice in the microbial fermentation sector. They can initiate food fermentation by producing acidic metabolites and other biomolecules due to their proteolytic activities (Lavermicocca *et al*., 2021). LAB are also known for its many advantages, such as extending shelf life, improving food safety, enhancing sensory, and possessing excellent properties that enable them to be used as probiotics (Li and Cao, 2010). Hence, they have been widely reported as a great biotechnological component that can improve overall quality and safety, particularly of fermented products (Ağagündüz *et al*., 2022).

Many LAB strains have been isolated from traditional fermented foods, and used to produce gamma-aminobutyric acid (GABA; Hasegawa *et al*., 2018). Some of the LAB strains known as GABA producers are *Levilactobacillus brevis* (Kim *et al*., 2022b), *Pediococcus pentosaceus*, and *Lactiplantibacillus plantarum* (Verni *et al*., 2022). GABA is a key bioactive compound produced by LAB (Ağagündüz *et al*., 2022). GABA is a four-carbon non-protein amino acid, with a molecular formula of $C_4H_9NO_2$, and produced from the reaction of l-glutamic acid by glutamate decarboxylase (Luo *et al*., 2021).

GABA is mainly produced by plants, microorganisms, and animals, and serves various functions depending on the source (Li and Cao, 2010;

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Diez-Gutiérrez *et al*., 2020; Luo *et al*., 2021). It is formed by a reaction called α-decarboxylation of L-glutamic acid and glutamic acid decarboxylase catalysation (Vo and Park, 2019). In some microorganisms, the accumulation of GABA can improve their tolerance to environmental acid stress (Luo *et al*., 2021). GABA primarily functions as a major inhibitory neurotransmitter that delivers chemical signals to the mammalian central nervous system (Li and Cao, 2010; Hasegawa *et al*., 2018; Diez-Gutiérrez *et al*., 2020; Luo *et al*., 2021), and it has positive effects on the mammalian vascular and central nervous systems (Ağagündüz *et al*., 2022).

GABA can be produced in several ways in the industry, including *via* chemical synthesis, microbial production, enzymatic reaction processes, and plant enrichment (Luo *et al*., 2021). Many GABA-rich foods have been developed, such as tea, red mould rice, germinated wheat, soy, and rice germ (Li and Cao, 2010). GABA has become one of the main interests in the food industry for food manufacturing, such as in the production of dairy products, since dairy products contain casein that is rich in glutamic acid, which means GABA can also be found abundantly in dairy products (Valenzuela *et al*., 2019).

To our knowledge, most GABA studies have concentrated on factors that influence the optimisation of conditions to produce GABA through LAB (Rayavarapu *et al*., 2021; Kim *et al*., 2022a; Zhang *et al*., 2022). There are also studies on the production of GABA by LAB species (Wu *et al*., 2017; Cui *et al*., 2020). Several papers that have discussed the production of GABA from LAB are summarised in Table 1.

To date, few studies and research papers are directed toward the biosynthesis of GABA, as well as the health benefits of GABA, which promote the application of GABA in the food industry. Although many studies were conducted on the production of GABA by LAB strains, they are rarely compared to one another in terms of their GABA-producing abilities. Therefore, conducting a systematic literature review on this topic can offer a more comprehensive understanding of LAB GABA, enabling extensive application in the food industry to meet the increasing demand for GABA-rich foods.

Table 1. Related review articles on gamma-aminobutyric acid by lactic acid bacteria from 2012 to 2022.

Year	Theme	Topic	Reference
2022	Optimisation of GABA production	i) Isolation of GABA-producing LAB ii) Optimisation of GABA production *via* OFAT strategy iii) Optimisation of GABA production *via* RSM	Zhang *et al*. (2022)
2021	Microbial production of gamma-aminobutyric acid	i) Applications of GABA in various fields ii) GABA biosynthesis by microorganisms iii) Engineering strategies for improved GABA microbial production	Luo *et al*. (2021)
2020	Production of gamma-aminobutyric acid from lactic acid bacteria	i) GABA-producing LAB species ii) Biosynthesis of GABA in LAB iii) Improvement of GABA production by LAB	Cui *et al*. (2020)
2017	High gamma-aminobutyric acid production from lactic acid bacteria	i) Biofunctionalities of GABA and GABA-rich food ii) Diversity of GABA-producing LAB iii) Challenges for manufacturing GABA or GABA-rich food	Wu and Shah (2017)
2017	Recent advances in gamma-aminobutyric properties in pulses	i) GABA as bioactive compound ii) Effect of pulse processing on GABA iii) Extraction and identification of GABA iv) Physiological role of GABA	Nikmaram *et al*. (2017)
2012	Production of gamma-aminobutyric acid by lactic acid bacteria	i) GABA-producing microorganisms and their isolation sources ii) Factors affecting GABA synthesis iii) Potential application of GABA-producing microorganisms	Dhakal *et al*. (2012)

Materials and methods

Research questions

Due to its psychobiotic properties, GABA has been widely used in various industries, such as medicine (Sadaeng *et al*., 2020; Van Hugte *et al*., 2023) and agriculture (Fan *et al*., 2022; Li *et al*., 2022a). Likewise, a study revealed that within the food industry, GABA has been widely applied to produce health-functional foods (Lorizzo *et al*., 2024). However, most of the studies conducted on GABA in the food industry is mainly focused on producing GABA in particular foods, which do not apply to the whole industry. Hence, there is an urge to study the best GABA-producing strains, the biosynthesis process, and the health benefits of GABA for future reference. The present review thus intends to search and gather information about GABA by LAB from previous studies to narrow the study gaps. The research question guiding this systematic review process is "What are the highest GABA-producers and the best fermentation conditions to optimise GABA production by LAB, the factors and different approaches to enhance GABA biosynthesis by LAB, and how can GABA help to improve health when being incorporated into foods?"

Search strategy

A rigorous search strategy was applied in this systematic review to ensure the efficiency of the data search, and the accuracy of the information gathered. The authors used the Boolean operator strategy (Bramer *et al*., 2018) to expand or narrow the search. Figure 1 summarises the search process comprising four stages: identification, screening, eligibility, and quality appraisal.

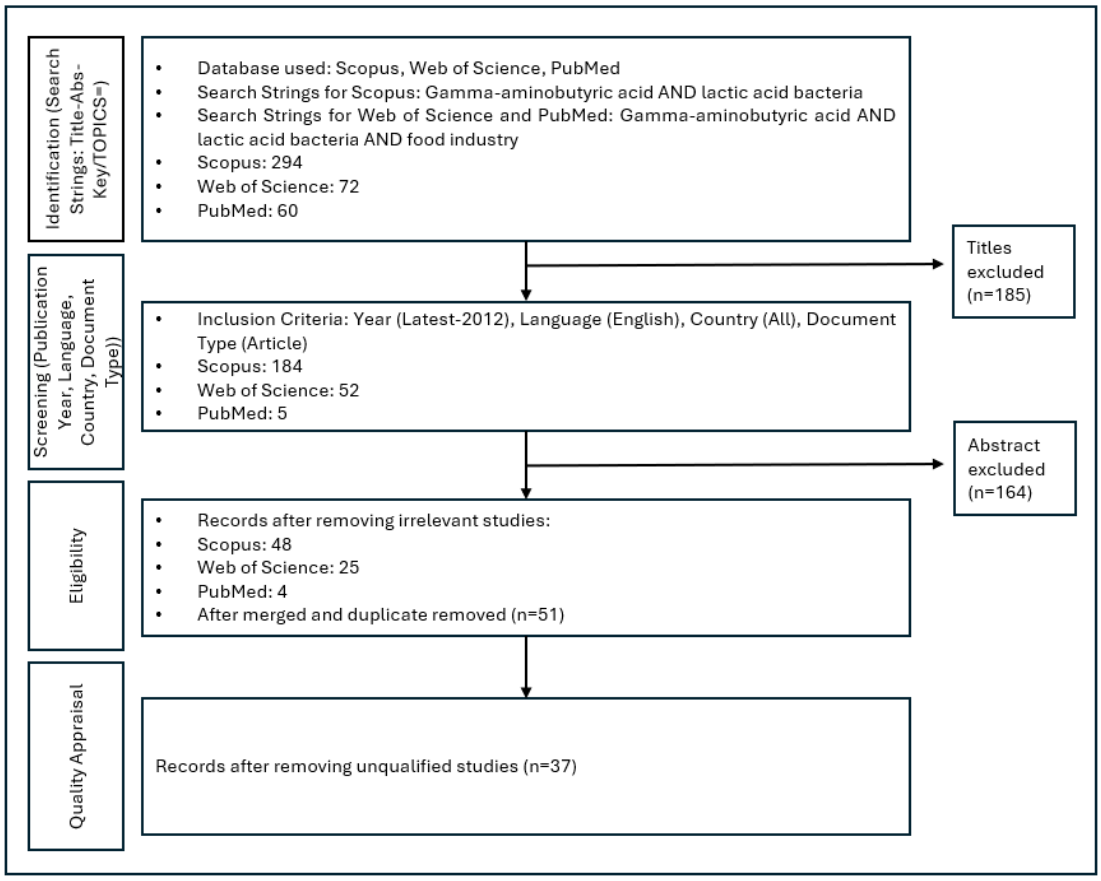

Figure 1. Schematic diagram of the searching strategy; *n* is the number of studies after the searching stage is conducted.

Identification

The identification process began on November 21, 2022, utilising three leading databases: Scopus, the Web of Science (WoS), and PubMed. All databases are subscribed by the library of University Teknologi Mara (UiTM), and affiliated with the authors. Scopus, WoS, and PubMed were chosen as they have extensive scientific literature that covers the fields and studies involved. The authors used the Boolean search strategy by incorporating the use of

the TITLE-ABS-KEY in Scopus, and TOPICS in WoS and PubMed, to find as many articles related to the topic as possible. The keywords used in this process were general terms associated with the title. This ensured that only relevant articles about the subject were chosen for the subsequent stage. At this stage, 426 articles were retrieved from the three databases: Scopus (294), WoS (72), and PubMed (60).

Screening

The next step after the identification process was the screening process, in which the search results were narrowed down and screened based on several inclusion and exclusion criteria. Similar inclusion and exclusion criteria were set for all databases to ensure a consistent result. The authors chose publications from 2012 to 2022 to ensure retrieval of the most up-to-date and relevant data to date. The authors limited the publication to only article types, as they are recognised as primary sources and original research. The authors included publications from all countries. However, the language was limited to only English articles. After the inclusion and exclusion processes, the authors obtained 184 articles from Scopus, 52 from WoS, and five from PubMed.

Eligibility

After the screening process, the authors proceeded with an eligibility check. At this stage, the authors skimmed through the titles and abstracts of the journals to ensure that only related studies were selected. As the present review focused on the application of GABA by LAB within the food industry in terms of GABA-producing strains, GABA synthesis, and health benefits, unrelated studies, such as optimisation of GABA production, GABA production by yeasts, and the application of GABA in the medical industry were excluded. Consequently, 48 results were obtained from Scopus, 25 from WoS, and five from PubMed. After merging and removing duplicates, the remaining articles that could be used in data extraction were 51.

Quality appraisal

The authors then assessed the remaining articles to choose the most relevant and legitimate studies. This process categorised the journals as high, moderate, and low quality. After this evaluation, only 37 were selected for use in the following process.

Data extraction

The authors constructed a table that organised the data from selected publications based on their title, findings, themes, and references to extract data from the selected articles. Articles and studies that focused on similar themes were grouped to make it easier for the authors to review the data.

Results and discussion

Background of studies reviewed

For this review, data from 37 chosen articles were extracted for thematic analysis. Three key aspects were identified by the authors, focusing on the applications of GABA in the food industry, which are discussed and reviewed herein. The oldest article chosen was published in 2012, and most of the studies were published in recent years, with eight articles in 2020 and seven articles in 2022.

Although there was no restriction when choosing the articles in terms of countries, some regions have conducted the most studies on GABA, with varying numbers of articles. This systematic review studied the amount of GABA produced by LAB strains in food fermentation, and the optimum condition for each strain. This topic might be helpful for future research as it includes a wide range of LAB strains that can produce high amounts of GABA in optimum conditions. Besides, this review also covers GABA biosynthesis to study the mechanism of GABA production in LAB, including the glutamate decarboxylase (GAD) genes involved and the health benefits of GABA when integrated into food-making to produce healthy and functional GABA-rich foods.

Themes

In this systematic literature review, three aspects of LAB GABA are reviewed: GABA-producing LAB strains from foods, GABA biosynthesis, and GABA health benefits. Each article that has been chosen refers to at least one of the themes.

GABA-producing strains

Twenty-three articles out of thirty-seven have discussed the production of GABA by different LAB strains. The articles reviewed the amount of GABA production by LAB strains that were isolated from Korean fermented foods (4), Chinese *paocai* (1), fish products (2), honeybees (1), black soybeans (1),

indigenous West Sumatera fermented food (1), Vietnamese marine products (2), sourdough breads (2), cheeses (2), Thai fermented foods (3), and various kind of beverages (4). These articles listed the amount of GABA produced by each LAB strain and the optimal conditions needed to achieve a high amount of GABA production. The selection of ideal LAB strains in optimum requirement is crucial in achieving high production of GABA in foods, especially when developing GABA-rich functional foods and beverages. Different kinds of LAB strains cultured in the most optimum conditions can produce different amounts of GABA.

In a study by Renes *et al*. (2017), 85 autochthonous LAB strains were isolated from cheese. However, only ten LAB strains were identified as GABA producers, coming from two major LAB groups: *Lactobacillus lactis* and *L. brevis*. All ten strains were incubated for 24 - 72 h to see whether the production of GABA could be increased. *L. brevis* TAUL195 showed the highest GABA concentration at 2524.05 µg/mL after 72 h of incubation in MRS-medium and 50 mg/mL of monosodium glutamate (MSG) that acted as the substrate. This showed that *L. brevis* strains are a better GABA-producer compared to *L. lactis.* Another study by Santos-Espinosa *et al*. (2020) isolated LAB strains from Mexican cheese, with 19 strains from *Lactobacillus* spp. and *Lactococcus* spp. producing GABA. Two strains from *Lactococcus lactis* (L-571 and L-572) produced the highest GABA concentration. However, after optimising the condition with incubation for 48 h at 37°C and 3 g/L of glutamate, the result concluded that *L. lactis* L-571 produced higher GABA concentration (1153.1 ± 13.5 mg/L) compared to the other strains.

Korean fermented foods, such as *kimchi* and *jeotgal*, are also popular choices for isolating GABA-producing LAB. A study by Park *et al*. (2013) showed that 75 LAB strains could produce GABA with over 50 µg/mL isolated from *kimchi*. These strains were further tested with different conditions, and as a result, strain K255 was selected as the highest producer of GABA (821.24 µg/mL) in MRS broth containing 3% MSG and 18 h incubation at 37°C. Strain K255 was later identified as *L. plantarum* K255. Lee *et al*. (2017) isolated 1,000 strains of LAB from *jeotgal*, which is Korean fermented seafood, and discovered *Enterococcus avium* M5 as the highest GABA producer of 13.85 ± 1.13 mg/mL when incubated in MRS medium with 3% MSG for 48 h.

There is also a study by Vo and Park (2019), who identified *L. lactis* LA43 as a new strain isolated from *jeotgal* that could produce the highest amount of GABA, 454 mM, among other isolated strains of *jeotgal*. They also did a simultaneous study on Vietnamese fermented fish products, and discovered that *Bacillus thuringiensis* LH2134 could produce up to 366.02 mM of GABA. Both strains were incubated in MRS broth supplemented with 1% MSG, and incubated at 30°C for 72 h. Kim *et al*. (2022a) also did a study on LAB-producing GABA by isolating LAB from various Korean fermented foods, and growing the bacteria in MRS broth with 50 mM MSG for 48 h at 37°C, and the result concluded that isolate FBT215, identified as *L. plantarum* FBT215, could produce high GABA content of 144.02 ± 14.40 µg/ml. Another study by Thuy *et al*. (2022) also discussed the production of GABA by 20 LAB strains isolated from *ruoc*, a fermented Vietnam shrimp paste. Isolate R13 showed the highest GABA concentration with 14.69 ± 0.166 mM when cultivated in MRS broth containing 1% MSG, and incubated for 24 h at 37°C. Isolate R13 was identified as *Lactiplantibacillus pentosus* R13.

A few studies have also been conducted on Thai fermented food. Kanklai *et al*. (2021) studied GABA production by LAB isolated from various kinds of Thai fermented food, such as fermented pork, beef, and fish. Of 36 isolates with the ability to produce GABA, only four isolates showed the ability as high GABA producers. The result indicated that strain F064A was the most efficient GABA producer of 2.85 ± 0.10 mg/mL compared to other isolates when incubated in MRS-MSG agar at 37°C for 24 h. Strain F064A, isolated from Thai fermented sausage was identified as *L. brevis* F064A. Another study conducted using Thai fermented food as a source of LAB isolation was done by Tanamool *et al*. (2020) using *plaa-som*, a fermented fish product. The strains were isolated in MRS agar for 72 h at 30°C and 4.0% of MSG to assess the highest GABA producer among 44 LAB strains isolated. The results showed that *L. plantarum* L10-11 produced 15.74 g/L of GABA, the highest among the isolates. On the other hand, a study by Sanchart *et al*. (2016) found another type of strain, which was *Lactobacillus futsaii* CS3, to be a high GABA producer isolated from *kung-som*, a different kind of Thai fermented shrimp, which could produce over 10 mg/mL of GABA after being incubated in MRS broth containing 20 mg/mL of MSG for 120 h.

Aside from Korean and Thai fermented foods, a study on indigenous West Sumatera fermented food by Anggraini *et al*. (2019) found 103 isolates of GABA producers from different sources. From 103 isolates, the DS15 strain showed the most efficient production of GABA isolated from curd, which produced 49.365 mg/mL of GABA in MRS broth containing 50 mM of L-glutamate, and incubated at 30°C for 72 h. Upon testing, the DS15 strain was confirmed as *Pediococcus acidilactici* DS15. Another fermented food studied for GABA-producing LAB was the Chinese *paocai*. Zhang *et al*. (2017) concluded that *L. plantarum* BC114 was the highest GABA producer among all the isolates as the GABA content recorded was 3.45 g/L when grown in MRS broth enriched in 20 g/L of MSG, and fermented for 72 h at 30°C.

Moreover, several studies have also used sourdough bread as a source of isolation for LAB. A study by Venturi *et al*. (2019) discovered 18 *Lactobacillus* strains capable of producing GABA isolated from various types of Italian sourdough bread, and the highest GABA-producing strain was *L. brevis* A7 at 136.62 ± 4.00 mg/kg of flour when incubated on MR3i broth for 6 h at 30°C. Likewise, Cataldo *et al*. (2020) isolated LAB from quinoa and amaranth sourdough. They found that *L. brevis* CRL2013 could produce a high GABA content of 266 mM when grown in MRS-GF broth, supplemented with 267 mM MSG for 72 h at 30°C.

Next, GABA-producing LAB has also been isolated from various kinds of beverages. Ohmori *et al*. (2018) used yogurt-sake to isolate LAB, and concluded that *Streptococcus thermophilus* A-1 was the highest GABA producer, approximately 40 times higher than other isolates found in yogurt-sake. This strain could produce GABA up to 3000 µM when incubated for 48 h at 37°C in MRS broth supplemented with 25% glycerol. Another study by Wang *et al*. (2021) used litchi juice as a source medium for the isolation of LAB, and discovered *L. plantarum* HU-C2W as the strain that could produce high GABA concentration at 3.92 g/L when grown on MRS agar supplemented with 4% MSG for 48 h at 37°C. Fermented almond and coix beverages were used as a medium source for GABA isolation in the study by Buatong *et al*. (2022). The result showed that the highest GABA concentration was produced by *L. plantarum* L42g at 496.7 µg/mL when grown in MRS broth enriched in 1% MSG and incubated for 72 h at 30°C. Li *et al*. (2016) isolated 377 strains of LAB-producing GABA from chickpea milk, and concluded that *L. plantarum* M-6 was the highest GABA-producer with 537.23 mg/L, and the optimum growing condition used 0.2% MSG, grown in MRS broth, and incubated at 39°C for 48 h.

Aside from that, studies have also focused on isolating GABA-producing LAB from marine seafood, such as shrimp and fish. Sakkaa *et al*. (2022) isolated 17 LAB strains from shrimp, and concluded that isolate SH9, also known as *Enterococcus faecium* SH9, was the highest GABA producer, producing up to 0.97 g/L of GABA when grown in an MRS medium containing 1% MSG for 48 h at 37°C. Wu *et al*. (2018) reported the production of GABA by LAB by isolating bacteria from fish samples. Their results showed that of 32 isolates, isolate RK03 (*L. brevis* RK03) could produce the highest GABA concentration among the isolates. *L. brevis* RK03, when cultivated in the optimum condition comprising MRS broth supplemented with 550 mM of MSG and incubation for 96 h, could produce 15,143 mg/L to 62,523 mg/L of GABA, with the addition of MSG and extended incubation period.

GABA was also produced in samples isolated from honeybees and black soybeans. Tajabadi *et al*. (2015) focused on honeybees as the source of LAB-producing GABA, and isolated 24 strains. Strain Taj-Apis362 was the highest GABA producer obtained from the honeybee sample, producing 7.21 mM of GABA at maximum, when grown in MRS broth containing 497.97 mM of glutamic acid, and cultured for a total of 60 h at 36°C. This strain is known as *L. plantarum* Taj-Apis362. On the other hand, Shabbir *et al*. (2022) focused on isolating GABA-producing LAB from black soybeans. It was found that *P. acidilactici* US1 was the highest GABA producer among eight strains isolated, producing 1.216 ± 0.06 mg/g of GABA when cultivated in MRS broth supplemented with 20% glycerol at 37°C for 72 h.

From the studies, it can be concluded that different fermentation conditions, *i.e*., incubation period, incubation temperature, and concentration of MSG, will influence the amount of GABA content produced by LAB as they affect the GAD systems and GABA biosynthesis process of the bacteria. This is congruent with the study by Yogeswara *et al*. (2020), who mentioned that optimising fermentation conditions can enhance GABA biosynthesis and production. Table 2 summarises the studies on the selection of high GABA-producing LAB in their optimum production condition.

Table 2. Summary of GABA-producing strains from foods and their production conditions.

No.	Food	Strain	Optimum production condition	GABA content	Reference
1.	Cheese	*Lactobacillus brevis* TAUL195	MRS broth 72 h incubation 50 mg/ml of MSG	2524.05 µg/mL	Renes *et al*. (2017)
2.	Mexican cheese	*Lactococcus lactis* L-571	MRS broth 48 h incubation at 37°C 3 g/L of glutamate	1153.1 ± 13.5 mg/L	Santos-Espinosa *et al*. (2020)
3.	*Kimchi*	*Lactobacillus plantarum* K255	MRS broth 18 h of incubation at 37°C. 3% of MSG	821.24 µg/mL	Park *et al*. (2013)
4.	*Jeotgal*	*Enterococcus avium* M5	MRS medium 48 h of incubation 3% of MSG	13.85 ± 1.13 mg/mL	Lee *et al*. (2017)
5.	*Jeotgal*	*Lactobacillus lactis* LA43	MRS broth Incubated for 72 h at 30°C 1% of MSG	454 mM	Vo and Park (2019)
6.	Vietnamese fermented fish	*Bacillus thuringiensis* LH2134		366.02 mM	
7.	Korean fermented food	*Lactobacillus plantarum* FBT215	MRS broth Incubation for 48 h at 37°C 50 mM of MSG	144.02 ± 14.40 µg/ml	Kim *et al*. (2022a)
8.	*Ruoc* (fermented shrimp paste)	*Lactiplantibacillus pentosus* R13	MRS broth Incubated for 24 h at 37°C 1% of MSG	14.69 ± 0.166 mM	Thuy *et al*. (2022)
9.	Thai fermented sausage	*Levilactobacillus brevis* F064A	MRS-MSG agar Incubated for 24 h at 37°C.	2.85 ± 0.10 mg/mL	Kanklai *et al*. (2021)
10.	*Plaa-som*	*Lactobacillus plantarum* L10-11	MRS agar Incubated for 72 h at 30°C 4% of MSG	15.74 g/L	Tanamool *et al*. (2020)
11.	*Kung-Som*	*Lactobacillus futsaii* CS3	MRS broth Incubation for 120 h 20 mg/mL of MSG	10 mg/mL	Sanchart *et al*. (2016)
12.	*Dadih*	*Pediococcus acidilactici* DS15	MRS broth Incubated for 72 h at 30°C 50 mM of L-glutamate	49.365 mg/mL	Anggraini *et al*. (2019)
13.	Chinese *paocai*	*Lactobacillus plantarum* BC114	MRS broth Incubated for 72 h at 30°C 20 g/L of MSG	3.45 g/L	Zhang *et al*. (2017)
14.	Italian sourdough bread	*Lactobacillus brevis* A7	MR3i broth Incubated for 6 h at 30°C	136.62 ± 4.00 mg/kg	Venturi *et al*. (2019)
15.	Quinoa and amaranth sourdough	*Lactobacillus brevis* CRL2013	MRS-GF broth Incubated for 72 h at 30°C 267 mM of MSG	266 mM	Cataldo *et al*. (2020)
16.	Yogurt-sake	*Streptococcus thermophilus* A-1	MRS broth Incubated for 48 h at 37°C 25% of glycerol	3000 µM	Ohmori *et al*. (2018)

17.	Litchi juice	*Lactobacillus plantarum* HU-C2W	MRS broth Incubated for 48 h at 37°C 4% of MSG	3.92 g/L	Wang *et al*. (2021)
18.	Fermented almond and coix beverages	*Lactobacillus plantarum* L42g	MRS broth Incubated for 72 h at 30°C 1% of MSG	496.7 µg/mL	Buatong *et al*. (2022)
19.	Chickpea milk	*Lactobacillus plantarum* M-6	MRS broth Incubated for 48 h at 39°C 0.2% of MSG	537.23 mg/L	Li *et al*. (2016)
20.	Marine shrimp	*Enterococcus faecium* SH9	MRS medium Incubated for 48 h at 37°C 1% of MSG	0.97 g/L	Sakkaa *et al*. (2022)
21.	Fish	*Lactobacillus brevis* RK03	MRS broth Incubated for 96 h 550 mM of MSG	15.143 mg/L	Wu *et al*. (2018)
22.	Honeybees	*Lactobacillus plantarum* Taj-Apis362	MRS broth Incubated for 60 h at 36°C 497.97 mM of glutamic acid	7.21 mM	Tajabadi *et al*. (2015)
23.	Black soybeans	*Pediococcus acidilactici* US1	MRS broth Incubated for 72 h at 37°C 20% of glycerol	1.216 ± 0.06 mg/g	Shabbir *et al*. (2022)

GABA biosynthesis

To produce GABA on an industrial scale, understanding how GABA is first synthesised is crucial. Of 37 articles, nine discussed the synthesis of GABA. Two out of nine articles discussed the factors influencing the GAD system in GABA biosynthesis, while four articles focused on the arrangement and location of GAD genes involved in biosynthesis. Two studies showed that the presence of a regulator gene can affect the biosynthesis of GABA, and another two studies suggested that the biosynthesis of GABA can be manipulated through mutational approaches.

Wu *et al*. (2017) mentioned in their study that two major pathways are involved in GABA production. The first pathway is the Puu-ADC pathway, which involves the degradation of putrescine; and the second pathway is the GAD pathway, *i.e*., the decarboxylation of glutamate. However, in bacteria, the decarboxylation of glutamate is more common compared to the degradation of putrescine, as the Puu-ADC pathway is absent. In LAB, GABA is mainly derived from the GAD pathway (Wu *et al*., 2017). In the GAD system, GABA is synthesised through an irreversible α-decarboxylation of L-glutamate, catalysed by glutamic acid decarboxylase (GAD), which is a pyridoxal-5′-phosphate (PLP)-dependent enzyme from MSG (Shi *et al*., 2014; Tajabadi *et al*., 2015; Li *et al*., 2020; Yogeswara *et al*., 2020). The glutamate decarboxylase (GAD) enzyme is an intracellular enzyme typically found in both eukaryotes and prokaryotes, and they require PLP as a cofactor (Yogeswara *et al*., 2020). The decarboxylation of the GAD system in LAB bacteria is known to be associated with the acid resistance mechanism, as it helps bacteria encounter acidic stress by reducing the proton concentration in the cytoplasm (Yunes *et al*., 2016; Yogeswara *et al*., 2020). This acid resistance mechanism is crucial to protect the bacterial cells in an acidic environment, especially for LAB, as they require this mechanism to survive and colonise the acidic condition during the fermentation of food (Gong *et al*., 2020; Yogeswara *et al*., 2020).

In general, the GAD system consists of a GAD operon and two fundamental GAD elements, which are the glutamate/GABA antiporter (*GadC*) and two homologous inducible GAD enzymes (*GadA* and glutamate decarboxylase-encoding [*GadB*]; Wu *et al*., 2017; Gong *et al*., 2019; Yogeswara *et al*., 2020). This system supports the glutamate import and GABA export in bacteria simultaneously (Yunes *et al*., 2016). Yunes *et al*. (2016) reported that the synthesis and export of GABA in LAB mostly requires the presence of *GadB* and *GadC* genes. Meanwhile, Wu *et al*. (2017) revealed that the efficiency of the GAD system in LAB is influenced by the activity of the *GadC* antiporter and the two GAD enzymes. Nakatani *et al*. (2022) reported that

each LAB strain has two different isoforms of GAD genes, *GadB*$_1$ and *GadB*$_2$. *GadB*$_1$ is located far from the GAD operon. In contrast, *GadB*$_2$ is located adjacent to the GAD operon, which includes the transcriptional regulatory gene (*GadR*), *GadC*, and glutamyl-tRNA synthetase (*gts*) gene. The GAD operon is located on the chromosomes of the LAB species. However, the gene organisation of each GAD operon differs and varies among bacterial strains and species (Yogeswara *et al*., 2020).

Of all LAB species, *Lactobacillus* spp. are known as efficient GABA producers and are thus being studied the most. Nakatani *et al*. (2022) has investigated *L. plantarum* kb1253, as this is the first strain of *L. plantarum* to have two GAD genes, including *GadC*. *L. plantarum* kb1253 was cultivated for 16 h, and the GAD activities were recorded. The result of the study showed that *GadB*$_1$ expression of *L. plantarum* kb1253 was the highest at pH 3, *i.e*., 2.8-fold higher than at pH 4. However, at pH 5, no expression was detected. Meanwhile, the expression level of *GadB*$_2$ increased as pH decreased, and like *GadB*$_1$, there was no expression detected at pH 5. Nakatani *et al*. (2022) also studied the effect of temperature on GAD activities and found that low temperature poorly affected *GadB*$_1$ activity, as little expression was recorded at 30°C. However, at 40 - 45°C, *GadB*$_1$ exhibited high expression, with 40°C being the most optimum temperature. Contrary to *GadB*$_1$, *GadB*$_2$ recorded little expression at temperatures above 45°C. Through this study, it is concluded that the presence of GAD operon comprising *GadB*$_1$ and *GadB*$_2$ led to high GAD activity, ultimately producing high GABA levels. Hence, *L. plantarum* kb1253 is postulated to possess a high GABA-producing capability due to the unique constitution of genes.

Next, several studies have been conducted on *L. brevis* due to its unique GAD system. Gong *et al*. (2020) performed a study on *L. brevis*, focusing on the presence of a nitrogen regulator in *L. brevis*. GlnR is a nitrogen regulator present in *L. brevis*, involved in the acid resistance mechanism, and functions as a direct modulator for GABA conversion from glutamate. Through the study, Gong *et al*. (2020) discovered that GlnR will inhibit the transcription of the *GadCB* operon, which involves genes such as *GadB*, *GadC*, glutamine synthetase-encoding gene *(GlnA*), and specific transcriptional regulator-encoding gene (*GadR). L. brevis* use the GAD system to maintain intracellular pH, and attain high GABA production, and the inactivation of the *GlnR* gene will increase the efficiency and capability to produce GABA. Based on the result demonstrated by Gong *et al*. (2020), the expression of *GlnR* was downregulated, while the expression of *GadCB* operon was upregulated in *L. brevis* ATCC 367, which produced 9.8-fold higher GABA compared to the wild type strain. The inactivation/deletion of *GlnR* can increase the transcription of the GAD system, and increase the acid resistance capability in bacteria, hence improving the conversion of GABA from glutamate.

Another study by Gong *et al*. (2019) studied the GAD system in *L. brevis*, which discovered that *L. brevis* has two GAD encoding genes (*GadA* and *GadB*) that share approximately 50% of protein sequence identity. The GAD activity is mainly operated by the *GadCB* operon activated by a transcriptional regulator *GadR* that regulates the activity in a glutamate-dependent manner. *L. brevis GadR* will activate the expression of the *GadCB* operon, which is highly correlated to the acid-resistance mechanism in the presence of glutamate. It can be concluded from the study that high expression of *GadR* genes led to efficient GABA production. Wu *et al*. (2017) also studied the distribution of genes in the GAD operon in *L. brevis.* They reported that all sequenced strains of *L. brevis* contained genes encoding glutamate decarboxylases (*GadA* and *GadB*), and an intact GAD operon (*GadR* regulator, *GadC* antiporter). It is the only species with a 100% probability of carrying an intact GAD operon in the chromosomes.

The *GadB* gene is located away from the GAD operon, while *GadA* is close to the *GadC* antiporter. This arrangement of *GadA* - *GadC* ensures the timely co-regulation of transcription and translation for GABA production, while the coexistence of *GadA* and *GadB* genes influences GABA synthesising capacity. Unlike *GadA* and *GadB* genes, the *GadC* antiporter only exists in certain species, indicating its strain-specific characteristics. GABA biosynthesis consumes cytosolic protons upon acid challenge, which is the reason behind the increase in the intracellular pH by glutamate, and this activates the *GadC* functions as well. Wu *et al*. (2017) concluded that the genome of *L. brevis* is unique to other LAB strains in terms of GABA production in two aspects: the intact GAD operon and the presence of GAD genes (*GadA* and *GadB*) encoding for glutamate decarboxylase. This study is consistent with another

study by Yogeswara *et al*. (2020), who reported that *L. brevis* contains two GAD-encoding genes (*GadA* and *GadB*), which expressed GAD enzymes that share approximately 50% amino acid sequence similarity, and that *GadC* is only present in the genomes of certain strains. However, Yogeswara *et al*. (2020) mentioned that the arrangements of the GAD operon are different for each LAB species. The glutaminase gene (*gls3*) is located between antiporter $GadC_1$ and $GadC_2$, while *GadB* accompanies $GadC_1$. Interestingly, the high GABA production capacity in *L. brevis* NCL912 is linked to the formation of complex *GadA* locus with *GadC*, enabling the coordinated expression of GAD genes and antiporter. Yogeswara *et al*. (2020) concluded that GABA biosynthesis could occur in three ways: through whole-cell reactions, recombinant bacteria, and GAD purification. However, the use of the whole-cell reaction method may incur some drawbacks, such as the decrease in GABA yields during cultivation due to the conversion of GABA to succinic semialdehyde by the GABA transaminase enzyme. On the contrary, GABA biosynthesis can be enhanced by optimising fermentation conditions, such as regulating glutamate concentration, pH, and carbon source. It can also be enhanced through metabolic pathway engineering and mutational approaches, such as directed evolution and site-specific mutagenesis (Yogeswara *et al*., 2020).

The enhancement of GABA biosynthesis through a mutational approach is discussed further by Shi *et al*. (2014). The study aimed to broaden the active pH range of $GadB_1$ through random mutagenesis (directed evolution) and site-specific mutagenesis. The *t17i* and *d294g* mutations on the genes can improve the $GadB_1$ activity through random mutagenesis. Meanwhile, the study discovered that at pH 6, the mutants showed higher activity than wild type $GadB_1$ using site-specific mutagenesis. This showed that site-specific mutagenesis is not as effective as random mutagenesis.

In a study by Li *et al*. (2020), the effect of different factors on GABA biosynthesis was discussed using whole-cell bioconversions of L-glutamate. The three factors studied were temperature, pH, and metal ions. The study on temperature showed that it influenced the conversion capacity by affecting the activity of the GAD system in bacteria, and different strains would have different optimum temperatures, which in turn would affect the optimum bioconversion rate as well. Next, to study the effect of metal ions, the author used *L. plantarum* 8014, and found that its bioconversion ability increased when the concentration of Zn^{3+} increased. Lastly, the effect of pH was also studied, in which the optimum pH of the conversion system was decided to be 7.5, since at this pH, bacteria could grow naturally, and L-glutamate could be completely converted without leaving any residue behind. Table 3 summarises the studies on GABA biosynthesis.

Health benefits of GABA

To incorporate GABA into the food industry as a health-functional food, assessment and analysis of its health benefits are necessary. This theme discusses several studies that show the proven health benefits of GABA in treating diseases that have been studied.

Several studies have mentioned GABA's wide range of physiological activities. It is said that GABA exists predominantly in the brain, and functions as a major inhibitory transmitter in the mammalian central nervous system (Li *et al*., 2016; Buatong *et al*., 2022; Sakkaa *et al*., 2022). According to Sakkaa *et al*. (2022), GABA can help regulate physiological diseases, such as Parkinson's disease, Alzheimer's disease, protein synthesis in the brain, and growth hormones, as GABA is essentially a psychobiotic compound. It is also found that GABA can synthesise and enhance the bioavailability of nutrients, boost the immune system, fight pathogenic microorganisms, and improve the human digestive tract. Furthermore, Buatong *et al*. (2022) stated that GABA has the effect of anti-hypertension, anti-carcinogen, anti-depressant, sedative, antidiabetic, and immunity enhancer. It is also stated that GABA, from the study on *L. plantarum* L42g strain-producing GABA, also has additional probiotic properties, *i.e*., tolerance to the gastrointestinal tract, co-aggregation with the most foodborne pathogen, and adhesion to the gut mucosa.

Another study by Li *et al*. (2016) reported that the lack of GABA or GABA deficiencies can cause neurological diseases such as Parkinson's and Huntington's diseases. Li *et al*. (2016) also mentioned that GABA could enhance the function of visual cortex cells, and improve the declining sensory, motor, and cognitive skills that occur as humans age. Their study of the extract of GABA-enriched fermented chickpea milk reported that 17.78 mg/g of GABA can induce a neuroprotective effect against cell death on manganese-induced PC12 cell death. In

Table 3. Summary of GABA biosynthesis.

Reference	Theme	Result
Gong *et al*. (2020)	Distribution of GAD operon	i) Presence of nitrogen regulator GlnR inhibited the transcription of GAD operon. ii) Inactivation of GlnR increased the transcription of GAD system and production of GABA.
Gong *et al*. (2019)	Distribution of GAD operon	GadR was the activator for GABA conversion which contributed to acid resistance, and activated the expression of GadCB operon essential for glutamate-dependant acid resistance and high GABA production.
Wu *et al*. (2017)	Distribution of GAD operon	*L. brevis* had different genetics than other LAB species in terms of GABA production, evidenced by intact GABA operon and two GAD-encoding genes (*GadA* and *GadB*), making it an efficient GABA producer.
Shi *et al*. (2014)	Mutational approaches to enhance GABA biosynthesis	i) Random mutagenesis on *t17i* and *d294g* genes enhanced the activity of $GadB_1$ gene. ii) Site specific mutation on bacteria showed higher activity of $GadB_1$ compared to wild type. iii) Random mutagenesis was more effective approach compared to site specific mutagenesis.
Yogeswara *et al*. (2020)	Mutational approaches to enhance GABA biosynthesis	GABA biosynthesis could be enhanced through mutational approaches, which are directed evolution and site-specific mutagenesis for higher GABA production.
Li *et al*. (2020)	Effects of different factors towards GABA biosynthesis	i) Temperature affected the GAD activity by influencing the conversion capacity of LAB. ii) Metal ion affected the bioconversion ability as GAD activity increased when ion concentration increased. iii) pH affected the conversion system in which optimum pH allowed bacteria to grow naturally and L-glutamate to be converted completely without leaving any residue.
Nakatani *et al*. (2022)	Effects of different factors towards GABA biosynthesis	i) $GadB_1$ activity was higher at low pH (highest at pH 3) while $GadB_2$ activity increased as pH decreased. ii) $GadB_1$ showed high activity at higher temperature while $GadB_2$ showed little expression at high temperature. iii) The presence of GAD operon including $GadB_2$ and *GadC* led to high GABA production.

the study, the cells underwent pre-treatment with FCE/GABA, which showed a significant increase of cell viability up to 26.80 - 48.80%, indicating that GABA protected the PC12 cell in a dose-dependent manner. The GABA effect on LDH activity was also studied. The result showed that GABA reduced the LDH activity (LDH activity - an indicator of cell membrane damage) of PC12 cells by attenuating the injury on the cell and retaining the integrity of the membrane.

Next, Zareian *et al*. (2015) performed a study on the effect of GABA on antihypertensive activity using spontaneously hypertensive rats (SHR). The result showed that norepinephrine (NE) components that elevate blood pressure were suppressed when GABA receptors in the hypothalamus were activated, and sympathetic outflow was regulated. The experiments on the SHRs found that when GABA was present, the plasma NE was reduced significantly, resulting in the modulation of vascular tone and ultimately causing the blood pressure to decrease. Zareian *et al*. (2015) also found several other GABA health benefits, such as GABA antioxidant activity that could reduce lipid peroxidation, thus allowing the SHRs to overcome the oxidative stress caused by hypertension, and the

modulation of the sympathetic nervous system by GABA which could decrease the vascular expression of ET-1 proteins in SHRs. The effect of GABA on angiotensin-l-converting enzyme inhibitory (ACE) activity, which increased to 38.4%, would produce a hypotensive effect.

Hussin *et al*. (2020) conducted a study on GABA to see its efficiency in the blood pressure-lowering activity using GABA produced by *L. plantarum* Taj-Apis362, a recombinant cell fermented in yogurt and fed to SHR. The authors found that the effective dose to induce a blood pressure-lowering effect was 0.1 mg/kg of GABA, and this result might have been due to the reduction of oxidative stress in SHRs. Thus, it was concluded that consuming GABA-rich yogurt by SHR upon single dose oral administration could cause lower blood pressure, and beneficial for antihypertensive studies.

Pouliot-Mathieu *et al*. (2013) also investigated the effect of GABA against hypertension. They experimented on the effect of cheese enriched with GABA on blood pressure in men with slightly elevated blood pressure. The cheese was produced using a starter culture containing *L. lactis* strains. In the experiment, 23 men with slightly elevated blood pressure consumed 50 g of cheese, equivalent to 16 mg of GABA, daily for 12 weeks. The results indicated that their systolic blood pressure decreased by 5.5 ± 3.9 mmHg, and the mean blood pressure decreased by 3.5 ± 2.8 mmHg. Although there was a decrease in blood pressure, the decrease was not much; thus, the authors concluded that 16 mg of GABA was not sufficient to significantly lower blood pressure, and suggested that consuming a higher dose than 16 mg of GABA could result in a greater decrease in blood pressure. The positive effects of GABA on lowering blood pressure have been proven by these three studies. The outcomes indicate the potential of GABA in the functional health food industry.

Subsequently, some studies highlight GABA's effect on human cognitive effect function. A study by Seo *et al*. (2012) investigated how GABA could improve long-term memory. The authors used *Lactobacillus sakei* B2-16 isolated from *kimchi*, which produced the highest GABA. About 46.69 mg/mL of GABA was fed to mice administered with scopolamine. The results showed that GABA improved the memory of the mice from 132 to 48 sec in a dose-dependent manner. However, this was only for long-term memory and not short-term memory. GABA induced protective effects against memory deficits, and additional consumption of GABA could enhance memory recovery by up to 85%. GABA also influenced the growth of PC12 nerve cells, which are in the pheochromocytoma cell line. Therefore, the experiment showed that a high dose of GABA was required to induce effective cognitive ability and recovery of brain damage in mice.

Another study by Shabbir *et al*. (2022) studied GABA produced by *P. acidilactici* US1 strain in fermented black soybeans. The authors found that GABA could treat neurological disorders, and assist the information-processing nerve to function properly while moderating the dysfunctional αβ effect in the hippocampus. It was concluded through this study that consuming GABA daily could improve or maintain cognitive function with verbal reasoning, working memory, and sustained attention in humans.

The effect of GABA on diabetic diseases is also studied. Abdelazez *et al*. (2022) did a study on the prevention of diabetic complications using GABA produced by *L. brevis* KLDS strains synthesised from camel milk. The authors found that GABA can successfully intervene against hyperglycaemia and hyperlipidaemia in streptozotocin-induced C57/6J mice, signifying the influence of GABA on hypoglycaemic activity indicated by reduced postprandial blood glucose levels, and a significant reduction in blood hypolipidemic and some liver enzyme levels. Abdelazez *et al*. (2022) also mentioned that consuming GABA as a medicinal health agent could reduce the inflammatory response, and prevent pre-diabetes development as GABA molecules have a regulatory effect on human diabetic islands by inhibiting the insulitis and systemic inflammatory cytokine production. Hence, GABA can regulate the blood glucose levels in mice, and prevent serious injury to the organs while improving the overall composition of blood plasma at the same time.

Nikmaram *et al*. (2017) also acknowledged the idea as they mentioned that GABA could reduce the risk of reverse type 1 diabetes (T cell autoimmunity) by inhibiting the inflammatory T cell response, preventing the stimulation of insulin secretion by a positive autocrine feedback loop in human pancreatic β-cells *via* GABA-GABA receptor system, as well as regulation of the replication and survival of pancreatic islet cells. It was also found that GABA could play a role in regulating glucagon release as

endogenous GABA from rat β-cells could inhibit the release of glucagon and insulin when GABA-GABA receptors were activated.

Another interesting study by Li *et al*. (2022b) found how GABA has an antiviral effect that can help prevent enterovirus infection, and relieve the symptoms of coronavirus infections while reducing the mortality rate. Li *et al*. (2022b) experimented on GABA produced by the *Lactobacillus fermentum* PV22 strain, which produced 45.757 ± 0.315 mg/mL GABA. The authors mentioned that GABA, an active antiviral compound, can offer protection against a wide range of viruses, from herpes zoster to the influenza virus. This mechanism works when GABA functions as an immunological molecule that transmits signals *via* the GABA "shunt" pathway, and modulates the production of hypoxia-inducible factor-1α and interleukin-1β. As glutamate decarboxylase protein has a highly similar structure to coxsackievirus and enterovirus, consumption of glutamate decarboxylase compounds can induce immunological surveillance against these viruses, thus reducing the risk of infection. The study revealed that *L. fermentum* PV22 could synthesise high levels of GABA in the minimum non-toxic dilutions (MNTD) associated with antiviral activities against murine norovirus (MNV). Thus, the probable antiviral mechanism of *L. fermentum* PV22 may be related to the effect of the GABA shunt pathway in macrophages against the virus. From this study, the author concluded that GABA may play an essential role in the inhibition of viruses. Table 4 summarises the study on the health benefits of GABA.

Table 4. Summary of GABA health benefits.

Reference	Benefit	Result
Li *et al*. (2016)	Neuroprotective effect against cell death	GABA can improve the function of visual cortex cells, alleviate the decline in sensory, motor, and cognitive skills; increase cell viability, attenuate cell injury, and retain the integrity of membrane cells.
Zareian *et al*. (2015)	Antihypertensive effect	Activation of GABA can suppress NE, which causes modulation of the sympathetic nervous system, regulate sympathetic outflow, reduce lipid peroxidation, increase ACE activity, and increase antioxidant enzymes.
Pouliot-Mathieu *et al*. (2013)	Antihypertensive effect	Consumption of GABA can decrease systolic and mean blood pressure when taken in doses higher than 16 mg.
Hussin *et al*. (2020)	Antihypertensive effect	GABA can decrease blood pressure by the reduction in oxidative stress.
Seo *et al*. (2012)	Memory improvement	GABA can improve long-term memory but not short-term, exhibit a protective effect against memory deficit, enhance memory recovery, and increase the growth of PC12 nerve cells.
Abdelazez *et al*. (2022)	Lower risk of diabetic disease	GABA can intervene against hyperglycaemia and hyperlipidaemia, and influence hypoglycaemic activity. GABA has a regulatory effect on human diabetics, and regulates blood glucose levels.
Nikmaram *et al*. (2017)	Lower risk of diabetic disease	GABA can reduce the risk of reverse type 1 diabetes, stimulate insulin secretion *via* GABA-GABA receptor, and regulate the replication and survival of pancreatic islet cells and glucagon release.
Li *et al*. (2022b)	Antiviral activity	GABA is an active antiviral compound that can provide protection against viruses, and function as an immunological molecule to transmit signals *via* the GABA shunt pathway, and modulate the production of hypoxia-inducible factor 1α and interleukin-1β.

Implications and limitations

This systematic review demonstrates GABA's high potential and implications in the food industry. Understanding the LAB strains' capability of producing a high yield of GABA, the process of GABA biosynthesis, and the potential health benefits

of GABA makes GABA a highly promising compound for incorporation into healthy functional foods. This finding can lead to new ventures in the fast-growing food market industry, targeting consumers who are looking for natural remedies or foods with multiple health benefits such as antihypertensive, antidiabetic, and stress relief. Researchers and industry players can strategically collaborate to conduct more in-depth studies on GABA to enhance and strengthen consumer trust in the credibility of GABA-rich functional foods. This will lead to further discoveries of GABA's benefits, and promote GABA-rich foods, thereby expanding the market reach and increasing GABA awareness among the public.

However, addressing a few limitations is still necessary to fully integrate GABA into the food industry. One limitation is the concern over GABA's bioavailability and stability. The concern over GABA's bioavailability and stability poses a constraint, necessitating the development of methods to enhance its stability during food processing and storage, ensuring it remains unaffected and maintains its expected efficacy and bioavailability. Another issue is the lack of standardised methods for GABA quantification, and the ambiguity surrounding its health claims. The public's scepticism about GABA's health benefits creates a barrier to gaining consumer trust and successfully entering the market. Therefore, we need to establish clear guidelines and regulations, along with wide-ranging promotions, for GABA-enriched foods to promote industry growth and cultivate positive public perception.

Conclusion

All 37 articles related to the topic were extracted from three databases, and discussed further in the present review. The present review is significant for the application of GABA in the food industry as it highlights the potential strains that can be used in foods, the synthesis of GABA by LAB, and the consumption benefits of GABA.

Although the present review discusses GABA-producing strains, biosynthesis, and health benefits, it is suggested and recommended to discover newer GABA-producing LAB strains that could produce a high yield of GABA with low safety risks, and understand more about GABA biosynthesis so that they can be manipulated and engineered to produce a favourable result. Another suggestion is to explore the synergistic effects of GABA with other bioactive compounds in order to identify novel therapeutic combinations. The potential of GABA as an antiviral compound was previously identified. Further research is warranted to develop more health-functional foods that address a wider range of health concerns.

Furthermore, although the production of GABA by LAB is one of the ways to yield GABA, studies on how to produce GABA through other alternatives must be explored for its wide implementation in the industry. The questions on why different strains of LAB produce different amounts of GABA also need to be resolved, which serve as research gaps for future research.

In summary, the application of GABA in the food industry is a potential field that can offer economic and health benefits to society. However, fewer studies have been done on GABA compared to other LAB components. Therefore, research on GABA must continue for it to be used widely in industry in the future.

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