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Identification, extraction, purification, and application of gamma-aminobutyric acid (GABA) in foods.

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I. Abstract

Gamma-aminobutyric acid (GABA) is a non-protein amino acid composed of four carbon atoms. GABA represents a point of convergence between traditional food science and modern health and wellness While gamma-aminobutyric acid is produced naturally by the body, this production process decreases as one ages. This implies that GABA can be taken as a supplement food. GABA is produced in many organisms including plants, animals, and various microorganisms such as bacteria, fungi, and yeast. Some actions of gamma-aminobutyric acid include enhanced sleep, decreased blood pressure, glucose reduction, relaxation, and neuroprotection. It is clear from the above analysis that the increased interest in GABA production worldwide is based on an intention to increase food safety and improve human health status. Lactic acid bacteria form one of the key groups responsible for GABA production. These bacteria assist in preventing food decay, fighting pathogenic bacteria, and stimulating the growth of beneficial microflora in the digestive system. From the viewpoint of food industry applications, GABA shows its huge capabilities for enriching functional properties of food while preserving all other sensory qualities and technical parameters. An example of such application is the use of GABA in the development of functionally enriched aged cheese.

Keywords: γ-Aminobutyric acid, food application, benefits, resist pathogens.

II. Introduction

In recent decades, the food industry has witnessed a remarkable evolution in consumer awareness of the close relationship between food and health. This has led to a global trend toward developing food products with added functional value, capable of promoting overall health and preventing various physiological disorders. This trend has contributed to highlighting bioactive compounds of natural origin, particularly those produced through microbial fermentation processes, due to their food safety and high applicability in industrial practice (Jena & Choudhury, 2024).

Lactic acid bacteria are a group of beneficial microorganisms known for their health-promoting properties. They are known for their ability to produce large quantities of biologically active compounds during fermentation.

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These compounds include lactic acid, peptides, exocytic polysaccharides, and enzymes such as lactase and protease. The production of these compounds by the bacteria depends on the specific fermentation process used (Abdul Hakim *et al*., 2023; Niamah *et al*., 2024).

Lactic acid bacteria are among the most important tiny living organisms because they can naturally produce lactic acid during fermentation. They are considered safe for food use and are widely applied in the food industry, especially in dairy and cheese products. These bacteria can grow well in moderate environmental conditions and produce many useful compounds that improve both the taste and nutritional quality of food (Ushidee-Radzi, 2025).

Gamma-aminobutyric acid (GABA) is a natural compound that has become increasingly important in both nutrition and health. Gamma-aminobutyric acid (GABA) is used in the body as a neuromodulator to dampen neural activities in the brain. As such, various therapeutic benefits may be observed, which include the lowering of blood pressure, enhanced neurological transmission, lowering of stress levels, and possible prevention from some chronic diseases. With this in mind, studies have looked into ways on how to produce GABA using safer, organic means rather than relying on the use of chemicals that can prove to be expensive and hazardous (Ibrahim et al., 2024).

2. Lactic acid bacteria (LAB).

Lactic acid bacteria are microscopic organisms which naturally occur in various types of food like milk products, fruits, and vegetables. These organisms are important to the health of humans because they can be used in making nutritionally valuable food items like yogurt and cheese. They manufacture various nutrients that enhance the health of consumers by supplying them with vitamins and minerals. Lactic acid bacteria are believed to be safe and natural organisms that help in the preservation of processed foods (Bennani et al., 2017).

Many research studies have shown that the lactic acid bacteria are gram positive, are either rods or cocci shaped, negative for catalase reaction, can grow under acidic and low salt conditions, and mostly feed on carbohydrates as a carbon source. These bacteria could be either motile or non-motile, and never produce any spores (George et al., 2018; Kocabay & Çetinkaya, 2020). The lactic acid bacteria produce many byproducts such as organic acids, which assist in preserving food. Thus, these bacteria are highly valued in terms of nutrition due to their beneficial role in food processing (Behbahani et al., 2023).

Genetic diversity exists among lactic acid bacteria because each species is able to produce varying amounts of lactic acid. While some lactic acid bacteria strains are very active in producing large amounts of lactic acid, others are known to produce smaller amounts or need special environments to do so. Diversity of lactic acid bacteria forms the basis of microbial selection as researchers try to find the most efficient strains (Santos-Espinosa et al., 2020).

According to Pannerchelvan et al. (2023), research on acidogenic microbes entails a combination of theory and application. Insights regarding their structure, metabolism, and production methods could be used to produce

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functional foods. This knowledge would help in formulating an approach for the production of better-quality foods (Niamah et al., 2023).

Various studies conducted by scholars such as Ghazanfari et al. (2023) show that certain strains of lactic acid bacteria may act as probiotics. This suggests that they could be included in functional food to improve the gut microbiome, aid in digestion, and help maintain the appropriate intestinal flora. According to the definitions set forth by FAO/WHO, probiotics are described as live organisms, which have beneficial effects on the consumer's health (Alebooye et al., 2023).

According to Moor et al. (2021), lactic acid bacteria have the ability to decarboxylate glutamic acid to form γ-aminobutyric acid (GABA). In addition to the above point, Iorizzo et al. (2023) added to it by highlighting the fact that GABA formation does not depend solely on lactic acid bacteria. However, safety issues regarding other bacteria limit their use in foods directly. Therefore, the present study focuses on safe lactic acid bacteria which can be easily used in food processing industries without requiring complicated regulations.

Table 1. Factors affecting acid production by lactic acid bacteria and their general impact on production efficiency.

Factor	**Effect on Production**
Bacterial strain type	Determines enzymatic capacity and metabolic efficiency
Availability of glutamic acid	Increases the amount of acid formed
Temperature	Influences enzyme activity and growth rate
pH level	Regulates stimulation or inhibition of the biosynthetic pathway
Fermentation time	Controls the final concentrations of acid
Nutrient medium composition effect	it affects bacterial stability and activity

3.Gamma-aminobutyric acid (GABA).

The term gamma-aminobutyric acid, which is also known as GABA, refers to a biologically important non-protein amino acid. In contrast to traditional proteinaceous amino acids, GABA is considered a functional bioactive molecule because of the lack of any role in protein structure. The characteristic nature of GABA is derived from the binding of the amino group at the fourth carbon (γ) position with respect to the carboxyl group (Jena & Choudhury, 2024).

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GABA occurs naturally in different organisms, including human beings, animals, plants, and microorganisms. However, the function and the mode of action vary from one organism and environment to another. For instance, GABA is mainly involved in the regulation of neuronal functions in human beings, while in microorganisms, GABA acts as an end product of metabolism under stressful conditions. GABA production in microorganisms has been shown to occur in acidic stress and nutrient deficiency environments (Hou et al., 2025).

Nutritionally speaking, GABA falls in the category of functional components that can be incorporated into the food chain without requiring the use of any synthetic substances. The incorporation of GABA into food products can be accomplished through the use of metabolic activity in certain types of microorganisms, such as lactic acid bacteria. In this context, the use of GABA is especially significant for the production of fermented foods like aged cheese and yogurts, which rely heavily on metabolic activities that take place both during production and ripening (Zhou *et al*., 2024).

GABA possesses certain physical and chemical characteristics that influence its viability in the industry. For instance, it is highly soluble in water, is colorless and odorless, and relatively stable in slightly aqueous conditions. This makes GABA very suitable for dairy products as they consist of aqueous systems with proteins, sugars, and salts among other components. High solubility ensures that GABA can be incorporated in the food system and increases chances of its absorption by the body (Hou et al., 2025).

Gamma-amino butyric acid (GABA) is described by having a four-atom carbon chain, ending with a carboxyl functional group at one end. Unlike other proteinogenic amino acids, GABA has an amino group that attaches itself to the γ-carbon atom instead of the α-carbon atom. The difference in its structure causes changes in its physical attributes such as ionization, reactions, and ability to undergo metabolism pathways (Cha et al., 2023).

4. Identification and Charactirization of GABA in biological and food matrices.

A number of different approaches were applied in order to detect and estimate the quantity of GABA. All these methods have some unique features and procedures depending on GABA biosynthesis pathway and the organism that produces it. GABA detection and characterization should be considered an essential part of studies on GABA biosynthesis. The scientific evaluation of a microbial strain or cultivation conditions can hardly be regarded as completed without the accurate identification and estimation of the level of GABA in the culture medium or final product. Such an approach is especially significant when GABA is detected in complex matrices like milk and cheese, since various elements could distort measurement (Pencheva & Teneva, 2022).

The recent studies have focused on quantification of GABA with an objective to estimate its amount in addition to any fluctuations during the fermentation process or maturation phase. The quantification requires a sophisticated

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method that can distinguish GABA from similar substances having identical behavior and structure to each other. It is required to precisely measure GABA in order to compare production efficacy in various types of microorganisms or effects of environmental/production parameters on production of GABA (Xiao et al., 2024).

The authors Lestari & Alvarez, (2022) state that intrinsic properties of the food matrix play a major role in determining the most suitable diagnostic test to be used. Matrices consisting of high contents of proteins and fat like cheese require further preparation to eliminate any possible interference that might affect the analysis process. This involves initial stages of extraction, filtration, or chemical modification of some sort to obtain a pure extract of the acid.

Falah *et al.* (2021) employed High-Performance Liquid Chromatography (HPLC) to estimate GABA levels using a standard acid prepared at a concentration of 1.84 mg/mL.

Similarly, Lyu et al. (2018) successfully determined GABA by applying HPLC coupled with UV detection at a wavelength of 254 nm. Similarly, Zarei et al. (2018) employed HPLC with a specialized program, also using a C18 column, but with UV detection at a wavelength of 630 nm to determine GABA concentrations. Carafa et al. (2019) utilized UHPLC-HQOMS to quantify GABA in milk and ripened cheese. They produced cheese aged for 20 days and observed changes in the levels of GABA and glutamic acid during the ripening period.

In another study conducted on a group of grains and cruciferous vegetables, the presence of GABA was identified using High-Performance Liquid Chromatography (HPLC). The acid was quantified, with maximum concentrations reaching 2.94 and 2.86 µg/mL (Al-Taher & Nemzer, 2019). Table (2) illustrates the common methods used for the diagnosis and characterization of y-aminobutyric acid (GABA).

Table (2): Common Methods for GABA Diagnosis and Characterization.

Method type	**Main purpose**	**Key advantage**	**Note**
Simple qualitative methods	Preliminary detection	Fast and low-cost	Limited accuracy
Analytical separation methods	Quantitative estimation	High accuracy	Requires sample preparation
Spectroscopic methods	Structural confirmation	High reliability	Specialized instruments

5. **Extraction and purification of Gamma-aminobutyric acid (GABA).**

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The process of extraction and purification of GABA represents an advanced stage in applied studies (Figure 1). The aim here would be to isolate this substance from the fermentation environment and other components that accompany it, depending on its use for analysis or application within specific foods and industries. The elimination of proteins is viewed as an important process in the isolation of GABA from foods because proteins can interfere with the separation process and negatively affect accuracy during analysis. Such a process is often conducted by heating the mixture at low temperatures or using proper protein-precipitating substances without compromising the GABA, which could otherwise lead to the destruction of GABA (Li et al., 2022). Industrial applications of GABA, particularly in the field of functional foods, do not always need to have GABA at a very high level of purity. Often, it is sufficient to have a sample of GABA at a concentration that allows its use in functional foods without any problems related to taste or safety concerns. In research investigations, on the other hand, much higher levels of purity are required since the purpose of such experiments is to examine GABA biologically or use it as a reference substance (Li et al., 2022).

The process of purification utilizes several approaches aimed at improving the purity of GABA as well as distinguishing GABA from other substances having a similar chemical nature. The effectiveness of these approaches depends on the ability to control the conditions, such as pH, salt, and temperature, as all these aspects influence separation. Therefore, a balance should be achieved between the necessary purity, costs, and time involved in the processes (Jing et al., 2023). Figure below illustrates the main steps in extracting and purifying GABA.

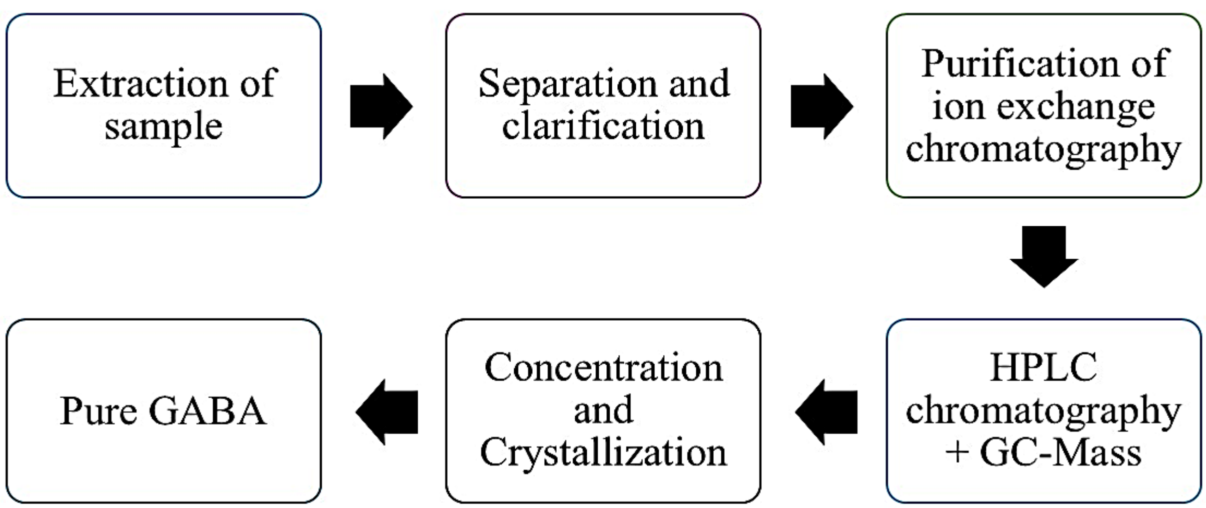

Figure 1. The basic steps of GABA extraction and purification.

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6. Alternative Methods for GABA Production.

The chemical method is considered one of the most ancient techniques used for producing gamma-aminobutyric acid (GABA). This technique uses chemical processes where chemicals undergo certain transformations resulting in the formation of GABA depending on controlled conditions of temperature, pressure, and catalysts (Table 3). The advantage of using this technique lies in the possibility to conduct mass production, although this technique usually faces various complications such as high expenses and complex technology, requiring the use of several purification stages due to formation of undesirable substances. Another drawback of this process is that there might be health risks when applying it to food products (Dhakal et al., 2012). Other than chemicals, there are strategies involving extracellular enzymes that have been devised. Such techniques take advantage of highly active enzymes in the transformation of suitable substrates to the target acid. These methods have higher accuracy than the chemical approach since they utilize specific enzymes in the transformation. However, the cost of implementing these techniques is often quite high because of the necessity of enzyme production, purification, and stability during production. Furthermore, for an industrial application, the process must be carried out under precise control (Heli, 2022).Some microorganisms other than lactic acid bacteria have also been employed for GABA production, utilizing different metabolic pathways capable of synthesizing the compound. However, the use of these microorganisms in food industries faces challenges related to food safety, which limits their application in products intended for direct human consumption (Yogeswara et al., 2020). It has been observed that comparing the different alternatives for GABA production shows that the choice of the most suitable method largely depends on the final production goal-whether for research, industrial, or food purposes (Table 3). While chemical or enzymatic methods may be appropriate for certain specialized industrial applications, biological production using lactic acid bacteria remains the most suitable option when integrating production into an existing food manufacturing process (Dhakal et al., 2012).

Table (3). General comparison between different alternatives for GABA production.

Production method	Main advantage	Key challenge
Chemical production	Large-scale quantitative yield	Cost and by-products
Enzymatic production	Precision and specificity	High cost
Non-food microorganisms	Sometimes high efficiency	Safety concerns
Lactic acid bacteria	Food safety and nutritional integration	Strain variability

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7. Application of GABA in food.

GABA-enriched foods include barley, chocolate, cheese, honey, rice, soy products, and fermented foods such as yogurt, cheeses, fermented vegetables, mung beans, and tea (Table 4) (Hinton & Johnston, 2020; Ngo & Vo, 2019). While interest in GABA is often attributed to its health benefits, this amino acid may also contribute to food quality. For instance, it plays a role in the functional properties of proteins. The presence of GABA has shown a pH-dependent effect on the crystallization characteristics of whey protein gel (Wang *et al*., 2019).

Recent developments in fermentation technologies using high-yield strains of lactic acid bacteria have significantly increased GABA concentrations, surpassing the baseline levels naturally present in raw materials. A wide variety of lactic acid bacterial strains isolated from traditional fermentation sources such as cheese, yogurt and fermented food have demonstrated their ability to biosynthesize GABA through the glutamate decarboxylase (GAD) pathway (Pannerchelvan et al., 2023). GABA is naturally present in small amounts in many plants, but it occurs at higher concentrations in fermented products, particularly fermented dairy and soy sauces (Diana et al., 2014).

Siragusa et al. (2007) were able to measure the acid concentration in cheese and identified acid-producing bacterial strains within it. These strains were isolated from 22 types of Italian cheese, where the acid concentration ranged between 0.26 and 391 mg/kg. Twelve strains of lactic acid bacteria from Italian cheese were capable of synthesizing the acid, and the strains with the highest acid production during the fermentation of reconstituted skim milk were, *Lactobacillus paracasei* PF6, *Lactobacillus delbrueckii subsp. bulgaricus* PR1, *Lactococcus lactis* PU1, *Lactiplantibacillus plantarum* C48 *and Lactobacillus brevis* PM17. May be marked differences in the GABA efficiency of various strains of a bacteria species, The GABA production capacity of different species is highly variable. Compared to other LABs, it has been reported that *Levilactobacillus brevis* can produce high amounts of GABA (205 g/L) (Wang et al.,2018).

The foods with the highest GABA content among pseudocereals are reported to be Tartary buckwheat and quinoa, with concentrations of 10.34 mg/100 g and 7.8 mg/100 g, respectively. This highlights the nutritional potential of pseudocereals as functional foods, since they naturally (Lee et al., 2023).

According to Bayat et al. (2022) the GABA content was increased to 19.9 mg/g in wheat germ as a result of the fermentation with *Lactiplantibacillus plantarum* 299v.

Saneto et al. (2020) reported that a quantity of GABA amounting to 86.2 mg/L was obtained during the production of fermented milk using strain from *Lactococcus lactis* L-571 and L-572 showed the highest production. Edalatian Dovom et al. (2023) succeeded in producing GABA at levels of 0.395 mg/mL when he used *Lactococcus lactis* 311 and 0.179 mg/mL *Lactococcus lactis* 491 during the production of Iranian traditional dairy products.

Table (4). Effect of lactic acid fermentation on GABA content in fermented dairy products.

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https://iasj.rdd.edu.iq/journals/journal/issue/20226 https://doi.org/10.54174/utjagr845

Fermented Dairy Products	Lactic Acid Bacteria	GABA Content	Reference
Fermented milk	*Lactobacillus paracasei* PF6, *Lactobacillus delbrueckii subsp. bulgaricus PR1, Lactococcus lactis PU1, Lactiplantibacillus plantarum C48 and Lactobacillus brevis PM17.*	0.26-391 mg /Kg	(Siragusa et al. 2007)
Brown rice	*Lactiplantibacillus plantarum, Lacticaseibacillus casei, Limosilactobacillus fermentum* and *Lacticaseibacillus rhamnosus*	6.93 mg/g	(Kwon et al.,2022)
Wheat germ	*Lactiplantibacillus plantarum* 299v	19.9 mg/g	(Bayat et al.,2022)
Fermented milk	*Lactococcus lactis* L-571 and L-572 showed the highest production	86.0 mg/L 86.2 mg/L	(Saneto et al.,2020)
Iranian traditional dairy products	*Lactococcus lactis* 311 and *Lactococcus lactis* 491	0.395 mg/mL 0.179 mg/mL	(Edalation et al., 2023)

8. Conclusion.

Overview of GABA in Agriculture and Food Industry from an Integrated Approach.

As seen in the comprehensive overview, it is clear that GABA is increasingly becoming relevant as a bioactive compound to agriculture engineering and the food industry, especially in today's world where there are efforts to develop functional foods that not only have nutritive values but can also provide certain health benefits. It is clear that GABA has certain chemical and biological characteristics that make it possible to produce biologically using lactic acid bacteria, whose advantage is their food safety aspect and ability to easily fit within conventional systems.

According to the literature review, the key importance of proper description and recognition of γ-aminobutyric acid (GABA) lies in the necessity to guarantee the reliability of the results obtained within any type of study, regardless of its nature and purposes. Additionally, the procedure of extraction and purification, despite certain variations depending on the desired aim of production, is still directly related to the physicochemical features of the substance and the peculiarities of the environment of production. The recent tendencies of the food industry indicate the preference for the extraction of GABA-containing extracts instead of obtaining the substance in its isolated form.

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https://iasj.rdd.edu.iq/journals/journal/issue/20226 *https://doi.org/10.54174/utjagr845*

In the context of food and health applications, the review reveals that the use of γ-aminobutyric acid (GABA) can significantly contribute towards adding value to food through the enhancement of its functional aspects, while at the same time not altering the sensory or technological properties of foods. The application of GABA in aged cheese is one such example where traditional foods have been converted to functional foods.

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