Recent advances in γ-aminobutyric acid (GABA) properties in pulses: An overview

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Abstract

Beans, peas, and lentils are all types of pulses that are extensively used as foods around the world due to their beneficial effects on human health including their low glycemic index, cholesterol lowering effects, ability to decrease the risk of heart diseases and their protective effects against some cancers. These health benefits are a result of their components such as bioactive proteins, dietary fibers, slowly digested starches, minerals and vitamins, and bioactive compounds. Among these bioactive compounds, γ-aminobutyric acid (GABA), a non-proteinogenic amino acid with numerous reported health benefits (*e.g.* anti-diabetic and hypotensive effects, depression and anxiety reduction) is of particular interest. GABA is primarily synthesized in plant tissues by the decarboxylation of L-glutamic acid in the presence of glutamate decarboxylase (GAD). It is widely reported that during various processes including enzymatic treatment, gaseous treatment (*e.g.* with carbon dioxide), and fermentation (with lactic acid bacteria), GABA content increases in the plant matrix. The objective of this review paper is to highlight the current state of knowledge on the occurrence of GABA in pulses with special focus on mechanisms by which GABA levels are increased and the analytical extraction and estimation methods for this bioactive phytochemical.

Keywords: Pulses; γ-aminobutyric acid (GABA); Glutamate decarboxylase; Health benefits; Processing

Introduction

For thousands of years, pulses have been considered as important dietary food products for human health around the world, which is mainly attributed to their high nutritional value, low cost and long shelf-life without cold storage.[1] According to the Food and Agriculture Organization (FAO), pulses are defined as "*Leguminosae crops harvested exclusively for their grain, including dry beans, peas and lentils*". Pulses are further categorized into 11 groups as follows: dry beans (including kidney, pinto, navy, adzuki, mung, black gram, scarlet runner, rice bean, moth, and tepary beans), dry broad beans (including the horse, broad, and field bean), dry peas, chickpeas, black-eyed peas, pigeon peas, lentils, bambara groundnut, vetch, lupins, and other "minor" pulses (jack, winged, velvet, and yam beans).[2–4]

Numerous studies have identified many associations between the consumption of pulses and health benefits; including the reduction of the risk of chronic diseases (*e.g.* obesity, diabetes, coronary heart disease, stroke, hypertension, and some types of cancer). The mechanism by which pulses may protect against diseases could involve the action of macronutrients (including resistant starch) and non-nutrient bioactive compounds (*e.g.* phytates).[5] From a nutritional perspective, pulses are good source of starch (including resistant starch), protein, dietary fibers, energy, minerals and vitamins, as well as different bioactive compounds.[6] Pulses contain various bioactive compounds such as phenolics, phytates, and oligosaccharides, which can play metabolic roles in humans and animals through a wide range of mechanisms of action.[7] Among these bioactive substances, γ-aminobutyric acid (GABA); a non-proteinogenic amino acid with several physiological functions and potential health benefits, will be the focus of this review.

There are many reports of health benefits of GABA including reduction of hypertension,[8] inhibition of chronic diseases associated with alcohol,[9] prevention of cancer cell proliferation,[10]

and modulation of blood cholesterol levels.[11] Microorganisms including lactic acid bacteria (LAB) and fungi (*e.g. Aspergillus nidulans*) have the ability to promote GABA production. For example, LAB has high cellular GAD enzyme activity. In this regards, GABA-producing LAB can be applied to develop fermented health oriented food.

It has been found that GABA is ubiquitous among plants and that its level in plant tissues is increased in response to stress conditions during plant growth, and during processing of the seeds such as soaking (*e.g.* in rice germ) and germination (*e.g.* in soybean).[12,13] Other processes that have been demonstrated to increase GABA concentration in plant materials are enzymatic treatment of wheat, gaseous treatment (*e.g.* bean sprouts such as soybean, black gram, green gram treated with carbon dioxide), pre-germination and fermentation (*e.g.* brown rice).[14] This review will focus on the composition of pulses with special emphasis on GABA as a bioactive compound with health benefits.

Pulses as an essential part of the diet

Composition and molecular characteristics

Pulses constitute an important part of the diet of the world's population. They contain high levels of complex available carbohydrates, proteins (of good essential amino acid balance), dietary fibers, vitamins and minerals, and low lipid content.[1] Of the legumes classified as pulses; the lupins stand alone with negligible available carbohydrate but very high levels of proteins and dietary fibers.[15,16] Within each species, there are differences in the reported composition depending on numerous factors such as variety, production environment as well as the techniques applied for nutritional analysis.[17] The protein content in pulses has been reported to range from 17% to 30%, with globulins and albumins as the major proteins, and prolamins and

glutelins as the minor proteins.[13] In spite of valuable levels of proteins, pulses are a relatively poor source of sulfur-containing amino acids (*e.g.* methionine, and cysteine). However, their lysine content is high in comparison with cereal grains.[18]

Pulses are rich sources of carbohydrates (50-60%) including starch, soluble sugars and dietary fibers. The soluble sugar fraction of pulses includes monosaccharides (ribose, glucose, galactose, and fructose) and disaccharides (sucrose and maltose). One of the main classes of oligosaccharides in pulses are the α-galactosides, where galactose is polymerized through α-D-1,6-linkages.[19]

Pulses contain many essential vitamins and minerals including substantial amounts of B-vitamins (*i.e.* thiamin, niacin, riboflavin, and pyridoxine) and minerals such as iron, calcium, potassium and zinc.[20] In general, they contain low levels of fat, varying from 0.83 g 100 g^{-1} for kidney beans to 6.6 g 100 g^{-1} for chickpea (Kabuli, India),[21] with lupin having higher levels.[16] Pulses contain variable quantities of compounds that can act as anti-nutritional factors including lectins, phytohemaglutinins and hemaglutininis, protease inhibitors, and phytic acid, which may interfere with the bioavailability of nutrients, with lupin having relatively low levels.[16] However, processing of these materials can help in lowering or removing these compounds prior to the consumption of pulses by either humans or animals.[22]

Nutritional and health aspects

Pulses are widely used as food and animal feed around the world due to their nutritional properties as well as their health benefits. Other non-nutrient bioactive substances found in pulses that are associated with cancer inhibition are saponins and protease inhibitors.[3] High amounts of other non-nutrient compounds in pulses, such as polyphenols and saponins, make

them a good food source for cholesterol lowering.[23] Moreover, the antioxidant ability of these compounds may lead to reducing the risk of heart diseases.[6] Due to the slow digestion of available carbohydrate in pulses, they are categorized in the low glycemic index (GI) food group (55 or less compared to white bread with GI of 100).[24] However, the GI of pulses is highly dependent on the pulse type and processing. For instance, Atkinson *et al.*[25] found that the GI of raw and canned chickpeas were of 10 and 38, respectively (compared to white bread with GI of 100). In addition, it has been reported that the *in vitro* rapidly available glucose levels (a predictor of high GI) of domestically cooked chickpeas was lower than that of commercially canned and commercially pre-cooked vacuum packaged chickpeas,[26] presumably due to greater heat exposure leading to increased gelatinization and breakdown of the starch. Lupin, however does not contain available carbohydrate (therefore cannot have its own GI value). However lupin addition to starchy foods such as white bread, has been reported to lower GI, possibly through its dietary fibers slowing the wheat starch digestion, and insulin stimulation by its proteins.[27]

The high-fiber content of pulses have been linked with lowering the risk factors of colorectal cancer and has been associated with an anti-proliferative activity.[28] The mechanisms responsible for this apparently protective role may include gene-nutrient interactions and modulation of protein expression. For instance, lupin kernel fibers when added to the diet for 28 days was reported to beneficially modify bowel function and putative fecal markers of colon cancer risk in a placebo controlled dietary intervention study of 38 healthy men.[29] Generally, high dietary fiber intake was also associated to decrease the blood cholesterol levels and limit the absorption of fats in the intestine.[30] Lupin kernel fiber addition to the diet has been demonstrated to provide a clinically beneficially reduction in cholesterol in a human dietary intervention study.[31] Due to their traditional use and numerous health benefits, pulses are included in various healthy diets

including Mediterranean diet, DASH (Dietary Approaches to Stop Hypertension) diet, and gluten-free diet.[28]

GABA as a bioactive compound

Chemistry and mechanisms of production

GABA is a four-carbon free amino acid, present in a wide range of microorganisms, plants and animals.[13] It is synthesized primarily by the decarboxylation of L-glutamic acid, referred as the GABA shunt that is catalyzed by glutamate decarboxylase (GAD, EC 4.1.1.15). In turn GABA can be converted to succinate semi-aldehyde by the mitochondrial enzyme GABA transaminase (GABA-T, EC 2.6.1.19) (Fig. 1).[12] GABA is a simple chemical substance with molecular formula $C_4H_9NO_2$, having a molecular weight of 103 Da.

PLEASE INSERT FIGURE 1 HERE

Biological methods of GABA production are more promising than chemical synthesis methods, since the former are mechanistically simple, have high reaction efficiency, and are environmentally "friendly".[33] There have however been many attempts to chemically or biologically synthesize GABA[34–36] because of its beneficial health functions giving rise to an increasing commercial demand.[37,38]

There are some suggested biosynthetic methods for efficient GABA production including immobilized cell technology,[33] sourdough fermentation (to make a high GABA functional bread),[39] and batch fermentation.[34,36,40,41] These techniques also have the potential to be applied for the production of GABA in the pharmaceutical and nutraceutical industries.

Physiological role of GABA

GABA has been reported to exhibit physiological roles such as the regulation of cardiovascular function[42] and reducing the risk of cardiovascular disease through modulating cholesterolaemia.[43] Due to such potential health effects, GABA has recently been applied in a wide range of functional foods, and nutraceuticals.[44] GABA was found to be effective in animal models to reduce the risk of reverse Type 1 diabetes (T cell autoimmunity) through inhibition of the inflammatory T cell response,[45] stimulation of insulin secretion by a positive autocrine feedback loop in human pancreatic β-cells via GABA-GABA$_A$ receptor system,[46] and regulation of the replication and survival of pancreatic islet cells.[47] Chen *et al.*[44] performed research to evaluate the anti-diabetic effects of GABA-rich yogurt on streptozotocin-induced diabetic mice. They reported that this functional food rich in GABA might improve hyperglycaemia and impaired glucose tolerance, as well as raising serum insulin concentration. Another animal model study obtained similar results in which GABA led to a rise in insulin secretion from the pancreas of normal rats.[48] Braun *et al.*[49] also confirmed the role of GABA in the regulation of glucagon release. They indicated that the release of endogenous GABA from rat β-cells inhibits the release of glucagon and insulin by the activation of GABA$_A$ and GABA$_B$ receptors, respectively.

The consumption of food with high GABA content was found to reduce the elevation of blood pressure and cholesterol in experimental animals,[50,50–52] as well as in humans.[35] Feeding spontaneously hypertensive rats with reduced-sodium soy sauce rich in GABA for 6 weeks resulted in lower blood pressure compared to a treatment of reduced-sodium soy sauce alone.[53] In another study, similar results confirmed the blood pressure-lowering effects of GABA by feeding spontaneously hypertensive rats with GABA-rich Chingshey purple sweet potato fermented-milk.[54] In contrast, Yang *et al.*[55] showed that the positive antihypertensive effect of

GABA was significant only during short-term (acute) administration, with an ongoing effect not observed during long-term (chronic) administration. It has however been reported by Inoue *et al.*[35] that a daily intake of 10 mg GABA (from fermented milk) for 12 weeks resulted in reducing the blood pressure by 17.4 mm Hg in hypertensive patients.

There are data supporting the important role of GABA intake in control of depression and anxiety. There is a positive relation between lower levels of GABA in cerebrospinal fluid and depression,[56] with several research works showing reduced level of GABA in the dorsolateral prefrontal and occipital cortex of depressed patients.[57–59] GABA-A receptors play a key role in modulating the different forms of anxiety, fears, phobias or depression.[60,61] Moreover, according to available data, GABAergic drugs (*e.g.* lorazepam) has indicated a close relation with anxiety-affecting properties,[62] since these drugs act as allosteric modulators of GABA receptors (also known as GABA analogues) and increase the available amount of GABA.[63] Other positive effects of GABA intake have been reported including diuretic and relaxation effects,[64] alcoholism treatment,[9] and raising of growth hormone level in plasma through an increase in the rate of brain protein synthesis in ovariectomized female rats.[65]

Extraction and identification of GABA

To determine GABA content of different food products, high performance liquid chromatography (HPLC) is considered as a powerful technique. Due to the weak UV-visible absorption characteristics of GABA, it requires derivatization before analysis with agents such as o-phthalaldehyde, 2-hydroxynaphthaldehyde, dabsylchloride, or 9-fluorenylmethyl chloroformate.[66] For example, mung beans, black beans, and soybeans were analyzed for GABA content using a modified method from Srisang *et al.*[67] GABA was extracted with 3%

sulfosalicylic acid (0.5 g 200 mL^{-1}). It was then analyzed by dimethyl-amino-azobenzene derivatization and HPLC using Supelcosil-LC-DABS column (acetonitrile was used as a mobile phase with a flow rate of 1 mL min^{-1}), and detected under visible light at 465 nm.[68] Other studies have also quantified GABA by HPLC in different materials including fermented lentils,[69] tea leaves,[42] brown rice,[70] kidney beans,[71] and soybeans.[72] In these studies, samples were derivatized with materials such as phenylisothiocyanate and o-phthaldialdehyde/2-mercaptoethanol.

Another method of GABA determination, carried out with an amino acid automatic analyzer, was described by Xu *et al.*[73] The basic principle of operation is the continuous flow chromatography procedure in which the sample is loaded onto a column of cation-exchange resin. In this method, free amino acid extracts (in protein hydrolysates or in native samples) were obtained after filtration through a 0.45 μm nylon syringe filter, and were analyzed by injection into amino acid automatic analyzer during a 50 min run. Amino acids were post-column derivatized with ninhydrin reagent and detected by absorbance at 570 nm. Identification and quantitation of GABA was performed by comparison to the retention time and UV spectra of authentic standards.

To detect GABA, biosensors have been developed. For instance, Niwa *et al.*[74] modified a glassy carbon electrode with bovine serum albumin-gabase-glutamate oxidase/osmium-poly (vinylpyrridine) by incorporating horseradish peroxidase. They reported the first on-line electrochemical sensor for the continuous measurement of GABA. Badalyan *et al.*[75] immobilized GABA-aminotransferase (GABA-T) and aldehyde oxidoreductase in a polymer containing an osmium complex on a graphite electrode. To fabricate the GABA-biosensor a premixed solution (including 2.5 μL of PaoABC (periplasmatic aldehyde oxidoreductase from *Escherichia coli*) (54 μM)), 2.5 μL of GABA-T (205 μM) and 1 μL of a freshly prepared PEGDGE (Poly (ethylene

glycol) (400) diglycidyl ether) solution (2.5 mg mL^{-1} in water) was placed on the top of the polished end of the 3 mm (diameter) polished spectrographic graphite electrode. Zhou and Muthuswamy,[76] fabricated an acoustic immunosensor by immobilizing a particular antibody on the gold surface of a quartz crystal electrode. The gold electrode surfaces were electrochemically characterized by using $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$ as the external redox probe. Although, these biosensors had good detection limits for sensing GABA at micro-molar levels, the construction of the electrodes was complicated and the biological substances incorporated into them were easily denatured under ambient conditions, which seriously hindered the stability of the electrodes.

Effect of pulse processing on GABA

Beans

Beans are considered as traditionally significant human foods with different types including broad beans (*Vicia* faba L.), wild beans (*Phaseolus vulgaris* L.), mung beans (*Vigna radiata* L.), and garbanzo beans (*Cicer arietinum*). Both conventional processing methods (*e.g.* heating) as well as modern processing technologies such as high pressure processing (HPP) have the potential to increase the bioavailability of bioactive compounds in food products. [77,78] Some of these technologies (*e.g.* fermentation) may increase the levels of GABA (Table 1).[79] Legumes are a good potential source for GABA production due to their high amounts of proteins. L-glutamic acid, the substrate for GABA synthesis, is one of the most abundant amino acids found in pulses such as faba beans,[80] and mung beans.[81]

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Faba bean (*Vicia faba* L.) is a commonly consumed legume rich in proteins, carbohydrates, and micronutrients. Recently, Coda *et al.*[82] investigated the effects of fermentation by *Lactobacillus plantarum* VTT E-133328 (30 °C for 48 h) of faba bean flour on GABA levels. A significant increase in GABA content, in all samples after fermentation, was observed.

It has been previously demonstrated that environmental stress during plant growth (*e.g.* salt stress) can increase the level of GABA in plants.[90] Yang *et al.*[91] reported that this phenomenon depends on stress intensity and duration. They reported a positive correlation between the level of NaCl treatment and GABA production in faba beans after 3 days, but a negative relationship was observed when the treatment increased to 5 days. These authors suggested that the longer stress treatment may have destroyed the activity of the enzymes responsible for GABA production (*e.g.* GAD and DAO).[91] The addition of a second stressor modified the level of GABA production in the faba beans. A combination of NaCl stress under hypoxia for 5 days resulted in a lower GABA level in the hypoxia-only control.

There are other factors that can enhance GABA production in pulses such as the plant hormone abscisic acid (ABA) (a stress hormone) that was found to raise GABA accumulation in faba beans under hypoxia-NaCl stress.[92] Other researchers have reported similar effects of ABA in common bean (*Phaseolus vulgaris*) demonstrating that ABA treatment (concentrations of 1 and 10 µM) resulted in the acceleration of GABA metabolism through lowering the negative effect of high NaCl concentration.[93] Other effective factors for modifying GABA production in pulses were reported to be Ca^{2+} and a chelating agent such as ethylene-diamine-tetra-acetic acid (EDTA). Calcium ions are required for GAD and DAO activation since these enzymes are Ca^{2+} binding proteins,[94] therefore Ca^{2+} addition (in $CaCl_2$ form) can lead to their activation, thus resulting in GABA content enhancement. In terms of GAD activity in pulses, environmental

conditions (e.g. pH and temperature) might also be important. For example, Wang et al.[95] found that to obtain 80% of GAD activity in rice bran, a pH range of from 5 to 9 and a temperature between 30 °C and 50 °C are essential. In contrast, EDTA chelates metal ions such as Ca^{2+}, and thus can through GAD and GAO inactivation lead to a reduction in GABA generation.[92] In support of this hypothesis, Yang *et al.*[96] reported direct correlation in fava beans between level of $CaCl_2$ treatment and both DAO activity and GABA production in the cotyledon and shoot. They also observed an inverse relation between EDTA-Na_2 level and DAO activity as EDTA inhibited GAD and DAO activities significantly thus inhibiting GABA accumulation. Another effective substrate that can influence on GABA content through GAD and DAO activity in legume seeds is L-glutamic acid (Glu).[97] It was observed by Guo et al.[97] that higher amount of Glu resulted in promotion of GAD and DAO activity; hence more GABA accumulation in the embryo and in the cotyledon of germinated soybean occurred.

During the polyamine degradation pathway in which GABA is synthesized, aminoguanidine (AG) acts as DAO enzyme inhibitor.[98] One study applied AG to germinating fava bean under hypoxia-NaCl stress to evaluate the functions of polyamine degradation pathway on growth and GABA accumulation.[99] It was reported that addition of 5 mM AG reduced DAO activity and GABA production of the sprouts as well as inhibiting their growth.[99] Other inhibitory factors of DAO are Mg^{2+}, Cu^{2+}, Fe^{3+}, EGTA (ethyleneglycol-bis (2-aminoethylether)-tetraacetic acid), L-cysteine, and β-mercaptoethanol. In addition, although Cu^{2+} is necessary for DAO molecular synthesis, an excess of Cu^{2+} leads to enzyme degeneration.[98]

There is variation between pulse species and between cultivars with a species in GABA content linked to variability in GAD activity. Oh *et al.*[100] reported different GABA content among several bean species including kidney, mung, wultari and adzuki, using rapid gas

chromatographic screening. The highest GABA level was found in adzuki bean, followed by wultari bean and mung bean. Li *et al.*[79] evaluated GABA accumulation among nine germinated fava bean cultivars, and concluded that smaller seeds had higher germination percentage, thus higher GABA content, since a significant negative correlation between germination percentage and 1000-kernel weight was observed. Moreover, cultivars with longer sprouts had higher GABA levels.

The conditions during seed germination have been demonstrated to affect GABA accumulation. Li *et al.*[79] observed a positive correlation between germination pH of fava beans and GABA level at a fixed temperature. They reported that the optimum pH value of germination was of 3.19 and under these conditions the GABA content in fava beans reached the maximum level. In addition, as the temperature of the germination process increased, a gradual increase in GABA accumulation was observed with a peak at 33.6 °C. These results are similar to those of Yang *et al.*[96], who reported an optimum germination temperature of 30 °C with a pH value of 3.0 for maximizing DAO activity of fava bean.

Mung bean (*Vigna radiata*) also known as green gram is commonly consumed in Asia. This bean has health related benefits including anti-inflammatory.[101] It was found that germination raised levels of GABA in mung bean to higher than that observed for fermentation (using *Rhizopus* sp. strain of 5351 inoculums under solid-state condition at 30°C for 48 hours).[83] The data of Mohd Ali *et al.*[84] showed a similar trend in which GABA content of mung bean was increased by about 28 and 7 times after germination and fermentation, respectively. Research work conducted by Tiansawang *et al.*[85] reported that germination of mung beans after 24 h of incubation lead to significant increase in GABA content to ≈0.8 g kg^{-1} dry matter. It should be noted that GABA accumulation is not distributed evenly throughout the seed. For instance, a

higher percent of GABA was found in the root tip than in the embryonic axis and cotyledons throughout the germination process.[102] Soaking and incubating mung beans for 0, 6, 12, 24, 36, and 48 h has been reported to significantly increase their content of GABA. The highest level of GABA was found after 24 h of incubation. In addition, it has been demonstrated that the cooking processes of boiling (98-100 °C for 20 min) and steaming (95-100 °C for 40 min) decreased GABA content in germinated mung beans, which was not observed with microwave cooking.[68]

Adzuki bean (*Vigna angularis*) is widely growing in Asia, and its consumption has been linked to several health benefits (*i.e*. reduced risk of heart disease and acetaminophen-induced liver damage).[103,104] GABA content in adzuki beans is very low (1.34 mg 100 g^{-1}), however soaking has been demonstrated to lead to a significant increase, with soaking temperatures of 35 and 45 °C giving maximum GABA levels (28.58 and 43.37 mg 100 g^{-1}, respectively).[86] Another study found a three-fold increase in GABA level for adzuki bean sprouts after 3 days seeding (63.29 mg 100 g^{-1}) in comparison with raw seeds (21.31 mg 100 g^{-1}).[87] Adzuki bean has been used as a medium for GABA-producing bacterial fermentation. In one report, GABA production using *Lactococcus lactis* and *Lactobacillus rhamnosus* fermentation of adzuki bean was investigated and the effects of immersion, germination, and cold shock (freezing temperature at -10, -20 and -80 °C for 24 h) before fermentation were evaluated.[86] The results demonstrated a 150 times increase in GABA level by using the cold shock treatment on the adzuki beans compared to the non-treated control.[86]

Kidney bean production has gained attention as a sustainable agriculture crop throughout Europe. Fermentation is known as a suitable method to enhance bioactive compounds of kidney beans. The type of microorganism applied for the fermentation plays a vital role in this process as recently investigated.[71] Two fermentation approaches of solid state fermentation (SSF) and

liquid state fermentation (LSF) were compared, each for 48 and 96 h. In SSF, *Bacillus subtilis* was used, whereas for LSF, spontaneous microorganisms found on the seeds (natural fermentation (NF) such as those of *Lactobacillus* genera or *L. plantarum* (LPF)) provided the fermentation.[105] The influence of different solutions (ascorbic acid, folic acid, glutamic acid, glutamic acid/chitosan, and lactic acid/chitosan) on the enhancement of GABA content in kidney beans has been also evaluated.[88] The results indicated that the highest GABA level of kidney bean sprouts was elicited by glutamic acid treatment (for 8 days).

Chickpea

According to FAO, chickpea (*Cicer arietinum* L.) is considered as the third most important grain legume in the world after beans (*Phaseolus vulgaris* L.) and peas (*Pisum sativum* L.), being widely grown in many subtropical regions.[2] In some Mediterranean countries, chickpea flour is used as a main ingredient for numerous traditional fermented foods, including sour dough fermented bread.[106] Coda *et al.*[39] conducted a study on twelve flours prepared from common wheat (*Triticum aestivum*), durum wheat (*Triticum durum*), rye (*Secale cereale*), spelt (*Triticum spelta*), oat (*Avena sativa*), buckwheat (*Fagopyrum esculentum*), rice (*Oryza sativa*), amaranth (*Amaranthus hypocondriacus*), millet (*Panicum miliaceum* L.), chickpea (*C. arietinum* L.), soy (*Glycine max*), and quinoa (*Chenopodium quinoa*), and then evaluated the effects of sourdough fermentation on GABA concentration. The highest GABA level was found in chickpea control dough (468±12 mg kg^{-1}) without bacterial inoculation. This high GABA level was attributed to the activity of endogenous GAD in flours. Two GABA producing bacteria strains *Lactococcus plantarum* C48 and *L. lactis* subsp. *lactis* PU1 were selected for fermentation, with higher GABA production being obtained when using *L. plantarum* C48. These authors also reported

that GABA concentration in the sourdough bread prepared from chickpea, buckwheat, amaranth and quinoa using *L. plantarum* C48, was significantly higher (504 mg kg^{-1}) than that for common wheat flour bread (made of wheat and baker's yeast) (11 mg kg^{-1}).

Lentil

According to Kuo *et al.*,[89] lentils (*Lens culinaris,* L.) after germination for 6 days showed an increase in the level of GABA up to 0.32 mg g^{-1} dry matter. Rozan *et al.*[107] reported that the GABA content of lentil was about 4 mg g^{-1} dry matter. Torino *et al.*[69] indicated that regardless of the fermentation system employed and microorganism type, GABA content of lentil increased during fermentation processing. They reported that the highest GABA level among different methods of fermentation, resulted from spontaneous liquid state fermentation (employing microorganisms already present on the seeds). This fermentation gave 10.42 mg g^{-1} GABA in the fermented lentil extract, compared with 7.16 mg g^{-1} extract for *L. plantarum* suspension and 6.54 mg g^{-1} extract for solid state fermentation with *B. subtilis*.

Conclusion

GABA is an important non-nutritive molecule found in pulses that shows great potential heath related benefits. Many factors influence the content of GABA in pulses including the type of cultivar, environmental stress during plant growth, and the processing method of the seeds (e.g. soaking, cooking, germination or fermentation). Treatment of plants with NaCl during growth (salt stress) appears to increase the GABA content in beans. However, it is necessary to carefully control these conditions as hypoxia can result in reduced GABA levels in faba beans. Fermentation process can be considered as a useful method to increase the levels of GABA in

pulses. In terms of other methods for GABA enhancement, emerging technologies such HPP are of great interest. There is now potential for the development of new functional foods from pulses with elevated GABA levels targeted at the whole population or for particular groups at risk of chronic diseases. However, before this becomes a reality, more research work needs to be conducted to develop commercially viable methods for the large-scale production of high GABA pulse seeds and pulse-based food products.

Acknowledgment

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Table 1. Effects of different processing techniques on GABA in pulses

Pulse type	Type(s) and condition of process	Main results	References
Faba bean (*Vicia faba* L.)	Fermentation: At 30 °C for 48 h with *Lactobacillus plantarum VTT E-133328*	There was a notable GABA content increase in all samples after fermentation.	Coda et al.,[82]
	Soaking and germination: At 28 ± 1 °C for 6 h in distilled water	- Higher germination percentage resulted in higher GABA content, and cultivars with longer sprouts had higher GABA levels. - The optimum pH value of germination for reaching the maximum level of GABA was of 3.19. By increasing the temperature of the germination process, a gradual increase in GABA accumulation was observed with a peak at 33.6 °C	Li et al.,[79]
Mung bean (*Vigna radiata*)	- Soaking: In chilled water at room temperature for 18 h before being steamed for 40 min - Fermentation: With *Rhizopus* sp. strain 5351 at 30 °C for another 48 h	Higher level of GABA was observed after germination compared to fermentation.	Yeap et al.,[83]
		GABA content of mung bean was increased about 28 and 7 times after germination and fermentation, respectively.	Mohd Ali et al.,[84]
Mung bean (*Vigna radiata*), soybean (*Glycine max*), black bean (*Vigna mungo*), and sesame (*Sesamum indicum*)	- Soaking: In distilled water (1:5, w/v) for 6 h at room temperature - Germination: For 48 h - Boiling: At different temperatures (98‑100 ºC) for 20 min - Steaming: In steaming pot for 40 min - Microwave cooking: 2450 MHz, 800 W for 10 min	- Germination (after 24 h) and soaking mung beans (for 0, 6, 12, 24, 36, and 48 h) led to significant increase the content of GABA. - The cooking processes of boiling (98-100 °C for 20 min) and steaming (95-100 °C for 40 min) decreased GABA content in germinated mung beans, which was not observed with microwave cooking.	Tiansawang et al.,[85]
Adzuki bean variety Kaohsiung No. 8 (*Vigna angularis*)	- Soaking: In 2500 ml of 0.7% sodium hypochlorite solution for 30 min at room temperature (25 ºC) - Germination: For 6 days - Fermentation: Using *Lactococcus lactis* and *Lactobacillus rhamnosus* at 37 °C for 24 h - Cold shock: At different freezing temperature (e.g. -10, -20 and -80 °C) for 24 h	- Soaking temperatures of 35 and 45 °C resulted highest GABA contents (28.58 and 43.37 mg 100 g^{-1}, respectively). - Application of cold shock treatment resulted in 150 times increase in GABA level compared to the non-treated control.	Liao et al.,[86]

Table 1. Continued

Pulse type	Type(s) and condition of process	Main results	References
Adzuki beans (*cv. Hongxiaodou* 1)	Soaking: In an artificial climate incubator at 25°C and humidity of 80% without sunlight and sprayed with water at intervals of 8 h every day	A three-fold increase in GABA contents after 3 days seeding (63.29 mg 100 g^{-1}) compared to the raw seeds (21.31 mg 100 g^{-1}) was reported.	Li et al.,[87]
Kidney beans (*Phaseolus vulgaris* var. Pinto)	- Soaking: In 0.07% sodium hypochlorite solution (1:6 w/v) for 30 min at room temperature Germination: in the darkness for 4, 6 and 8 days at 20 ºC - Elicitors: In distilled water at the following concentrations: 500 μM ascorbic acid; 50 μM folic acid; 5 mM glutamic acid; 50 ppm low-molecular weight (LMW) chitosan in 5 mM glutamic acid; 50 ppm LMW chitosan in 5 mM lactic acid	The highest GABA level was elicited by glutamic acid treatment (for 8 days).	Limón et al.,[88]
Chickpea (*C. arietinum* L.)	Fermentation: For 24 h at 30 °C with *Lactobacillus plantarum C48* or *Lactococcus lactis* subsp. *lactis PU1*	- The highest GABA level was found in chickpea control dough (468±12 mg kg^{-1}) without bacterial inoculation. - Higher GABA production was obtained using *L. plantarum C48*.	Coda et al.,[39]
Lentils (*Lens culinaris,* L.)	- Soaking: In 2500 ml of 0.07% sodium hypochlorite solution for 30 min at room temperature - Germination: On a pilot scale, by layering seeds over a moist filter paper, continuously watered by capillary in a seed germinator for 2, 4 and 6 days with continuous light	Germination for 6 days resulted an increase in the GABA content up to 0.32 mg g^{-1} dry matter	Kuo et al.,[89]
	- Liquid state fermentation: Either spontaneously with the only microorganisms present on the seeds or by inoculation of *L. plantarum* suspension (108 CFU ml^{-1}) at 1-2% (v/v) for 96 h at 37 °C - Solid state fermentation: Sterile cracked seeds were homogeneously inoculated with 5% (v/w) of *B. subtilis* (105 CFU g^{-1}) saline suspension, then incubation for 96 h at 30 °C and 90% humidity	- Regardless of the fermentation system employed and microorganism type, GABA content of lentil increased during fermentation processing. - Application of spontaneous liquid state fermentation (employing microorganisms already present on the seeds) provided the highest GABA level among other studied fermentation methods.	Torino et al.,[69]

Figure captions

Figure 1. Synthesis of GABA by GABA shunt. GAD: glutamate decarboxylase, GABA-T: GABA transaminase, SSADH: succinic semi-aldehyde dehydrogenase. (Adapted from Olsen and DeLorey,[32]).
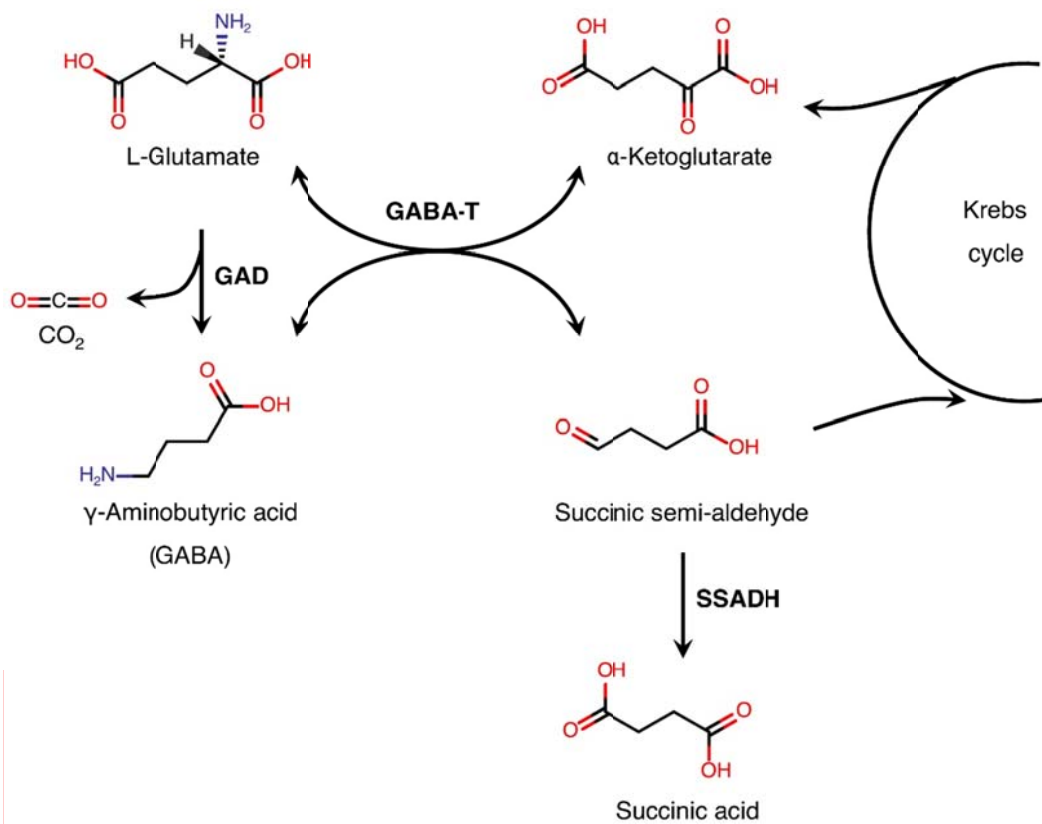

Figure 1.