Journal of Food Composition and Analysis 122 (2023) 105416

Contents lists available at ScienceDirect

Journal of Food Composition and Analysis

journal homepage: www.elsevier.com/locate/jfca

Screening for GABA and glutamic acid in tomato and potato genotypes and effects of domestic cooking

Tessa H. de Bie [a,b,*], Ric C.H. de Vos [b], Henriëtte D.L.M. van Eekelen [b], Frank F. Millenaar [c], Cindy K.M. van de Wiel [d], Josephus J.H.M. Allefs [d], Michiel G.J. Balvers [a], Renger F. Witkamp [a], Maarten A. Jongsma [b]

[a] Division of Human Nutrition and Health, Wageningen University & Research, P.O. Box 17, 6700 AA Wageningen, the Netherlands
[b] Wageningen Plant Research, Wageningen University & Research, P.O. Box 16, 6700 AA Wageningen, the Netherlands
[c] BASF's Vegetable Seeds Business, Napoleonsweg 152, 6083 AB Nunhem, the Netherlands
[d] Agrico, Duit 15, 8305 BB Emmeloord, the Netherlands

ARTICLE INFO

Keywords:
GABA
Glutamic acid
Food
Tomato
Potato
Screening
Cooking
Baking
Frying

ABSTRACT

γ-Aminobutyric acid (GABA) and its precursor glutamic acid play signaling roles in both humans and plants. Interestingly, positive effects on human health are ascribed to GABA consumption, which is present at relatively high levels in various food products, including potato tubers and tomato fruits. However, the currently available information on GABA content in foods only partly represents market categories and lacks data on glutamic acid. Here, we performed a screening of 98 tomato and 72 potato genotypes for GABA and glutamic acid levels. Our results show a large variation in both GABA and glutamic acid across the various genotypes. The GABA and glutamic acid levels ranged from 72 to 1122 μg/g fresh weight (FW), and 1160–6513 μg/g FW, respectively in tomato, and were between 68 and 759 μg/g FW and 409–874 μg/g FW in potato. Differences between market categories were only present for glutamic acid. For both GABA and glutamic acid, losses occurred with cooking, depending on the preparation. GABA was less affected by cooking than glutamic acid. Potato and tomato could be major dietary GABA sources. Especially high-GABA genotypes merit further investigation because of their potential health effects.

1. Introduction

γ-Aminobutyric acid (GABA) and its precursor glutamic acid are amino acids present at up to gram per kilo levels in many food products. They are either endogenously produced in for example melon, tomato and potato or generated during fermentation in for example matured cheese, fermented soy products and sauerkraut (Herawati et al., 2021; Nakamura et al., 2006; Redruello et al., 2020; Saito et al., 2008; Toyoizumi et al., 2019; Yang et al., 2020).

In plants, glutamic acid is incorporated into proteins and involved in amino acid metabolism as a precursor for (among others) GABA, and both molecules are involved in diverse physiological, defense, signaling and reproductive processes (Forde and Lea, 2007; Toyota et al., 2018). GABA is directly toxic to pest insects, and for this reason, it is presumed to be constitutively present in high amounts specifically in some fruits and storage organs like tomato and potato tubers (~500 μg/g) (Bown and Shelp, 2016; Ramesh et al., 2017; Scholz et al., 2015). Yet, in tomato fruits, the high content of glutamic acid introduces an umami flavor which is assumed to enhance fruit consumption by birds and mammals (Takayama and Ezura, 2015). This promotes seed dispersal as tomato seeds survive digestion and will germinate from fecal deposits.

In human biology, both glutamic acid and GABA are primarily known as neurotransmitters in the central nervous system. At the same time, oral supplementation with GABA has been shown to have metabolic health effects in rodents (Hayakawa et al., 2004; Tian et al., 2011). Most research on the health benefits of GABA in humans has been performed with supplemented GABA (Li et al., 2015). GABA supplements are widely available, claiming to improve sleep and reduce stress. Recently, we determined that GABA bioavailability is not influenced by a tomato food matrix (Bie et al., 2022). This suggests that an increased intake of GABA, through the consumption of tomato fruits or potato tubers that are relatively high in GABA for example, could lead to

* Correspondence to: Stippeneng 4, 6708WE Wageningen, the Netherlands.
E-mail address: thdebie@gmail.com (T.H. de Bie).

https://doi.org/10.1016/j.jfca.2023.105416
Received 2 March 2023; Received in revised form 26 April 2023; Accepted 21 May 2023
Available online 24 May 2023

positive health effects.

Tomato and potato are staple foods in many cultures. For instance, the consumption of fresh tomatoes in the EU is currently 15 kg per capita, and an additional 18 kg of processed tomatoes is consumed (Directorate-General for Agriculture and Rural Development., 2021). Potato is eaten in 82% of the world's countries and is one of the largest food energy suppliers. In 2013 the consumption of potatoes in the EU was 83 kg per capita, which is the largest volume in the world (Wijesinha-Bettoni and Mouillé, 2019). Consequently, they could provide a source of GABA in the diet, in particular when high GABA varieties are consumed and provided that preparation methods are compatible.

Next to GABA, the levels of glutamic acid are also relatively high in potato and especially in tomato (Skurray and Pucar, 1988). It is relevant to study both GABA and glutamic acid in crops, considering their biosynthesis is interconnected by for example the GABA shunt (Fait et al., 2008). While GABA is the most abundant free amino acid in green tomato fruits, its concentrations decrease substantially during ripening (Sorrequieta et al., 2010). On the other hand, the free glutamic acid concentration increases steadily during tomato fruit ripening and it represents the most abundant amino acid in red tomato fruits. Research investigating glutamic acid content of tomato fruits is mainly focused on increasing the characteristic umami flavor to which glutamic acid contributes (Brosnan and Brosnan, 2013).

Currently, there is considerable interest in increasing the GABA content of food products. A gene-edited tomato containing 1250 μg/g FW GABA is already marketed in Japan (Nonaka et al., 2017). In addition, relatively GABA-rich plant products can be obtained by specific growth conditions (e.g., salt stress (Zushi and Matsuzoe, 2007), selection among existing cultivars, or by means of breeding programs making use of the genetic variation in wild species (Gramazio et al., 2020). Some literature describing the GABA and glutamic acid content of different tomato and potato genotypes is already available. For GABA in tomato fruits, a screening of 61 genotypes was performed in a field trial by Saito et al. (2008), who found a 20-fold difference between genotypes in GABA content (ranging from 90 to 1000 μg/g) (Saito et al., 2008). For glutamic acid only a narrow selection of tomato genotypes has been investigated and the content range was not more than 2-fold (Baldina et al., 2016; Pratta et al., 2004; Tommonaro et al., 2021). For example, Pratta et al. (2004) studied the glutamic acid content in 5 different tomato cultivars and reported a variation from 1600 to 2800 μg/g FW (Pratta et al., 2004). For potato, Nakamura et. al (2006) analyzed the GABA content of tubers from 22 varieties and showed a range of about 3.5 fold, from 239 to 819 μg/g FW (Nakamura et al., 2006). However, these genotypes only partly represent the existing market categories and none of these screenings involved both GABA and its precursor glutamic acid (Dobson et al., 2008; Halford et al., 2012; Nakamura et al., 2006; Pęksa et al., 2021; Uri et al., 2014). Thus, a large representative screening of both GABA and glutamic acid in tomato or potato is still lacking.

Therefore, we aimed to determine the extent of variation in GABA levels in a representative range of tomato and potato genotypes. In the search for tomato and potato varieties with a high GABA content, we analyzed the levels of GABA and its precursor glutamic acid in 98 tomato and 72 potato genotypes and cultivars. To gain more insight, we also studied the variation in effect of fruit maturation on GABA content and the effect of harvest year. As tomato is frequently consumed after some form of processing and potatoes are not eaten raw, we also included an analysis of the effects of most commonly used domestic cooking methods on GABA and glutamic acid content.

2. Methods

2.1. Planting, harvesting and sampling procedures

2.1.1. Potato

A series of 72 genotypes, consisting of cultivars and breeding clones, were grown in clay soil in the seasons of 2017, 2018, and 2019 in Bant, Noordoostpolder, The Netherlands. Seed tubers had been produced and stored at that location in the previous year. The planting dates were in April on the 12th, 18th, and 17th in the three consecutive growing seasons, respectively. Plots consisted of 16 plants each, planted in two rows that were 75 cm apart. Planting was at a 33 cm distance within rows, with 4 border plants separating the plots of different genotypes. Fertilization was in total 180–190 kg of N per ha, including the soil stock of approximately 25 kg N per ha. Of this total N, 120 kg per ha was given in the form of a N:P:K mixture in a ratio 23:23:0 immediately after seed tuber planting, but before hilling, and the remaining N was supplied in the form of calcium ammonium nitrate after tuber initiation of the mature plants. Protection against plant diseases was carried out according to local agricultural practice. Harvest dates of mature tubers were October the 16th in 2017 and September the 12th and 19th in 2018 and 2019, respectively. Tubers were mechanically harvested and subsequently stored in boxes at ambient temperature (8–12 °C) in the dark for about 3 months until sampling for analysis.

Ten average-sized tubers were randomly chosen per box and washed with tap water. One wedge, approximately 0.5 cm width at its widest side, was cut from each tuber between the stolon and stem end. Wedges were quickly cut into smaller parts and frozen in liquid nitrogen for immediate processing or storage at − 80 °C. For a range of genotypes (Cumbica, Monika, Concordia, AR 12–5137, Hansa, Cerisa, Blue Star, Fontane, AR 91–1409, ARD 11–3181) the GABA and glutamic acid content was also assessed in peel and flesh separately. For this experiment, 10 tubers per genotype were selected. The cut wedges of 0.5 cm wide were peeled, the flesh was cut into small pieces and both flesh and peel were stored and processed separately. Tuber parts were then milled into a fine powder using a liquid nitrogen-cooled analytical mill (IKA, Staufen, Germany) and aliquots (100 mg, < 2.5% deviation) of the frozen ground powders were weighed in a 2 mL Eppendorf tube) and stored in 4 mL cryotubes at − 80 °C until analysis.

2.1.2. Tomato

A collection of 98 tomato genotypes, consisting of both cultivars and breeding lines, was screened. The cultivars represented both cluster (normal round and beef), snack, and cherry types of fruits. The breeding lines partly consisted of heirloom genotypes and elite lines. Seeds were sown in small rockwool plugs (Oct 27, 2017), transplanted in blocks (Dec 10, 2017) and finally transplanted to a greenhouse with rockwool slabs (Jan 4, 2018). The greenhouse was located in Roggel (Netherlands). Pruning of the trusses from plants in the cluster segment was done to 6 fruits remaining per cluster. Only fully ripe fruits were used in the genotype screening. Harvest started on March 14, 2018, and the fruits used for the measurements were harvested on May 21, 2018.

The effect of fruit ripening on GABA and glutamic acid levels was subsequently evaluated in 7 other tomato genotypes (cv. Madara, Marejada, Maremagno, Marinda, Marmarino, Micro Tom and Moneymaker). For this experiment, seeds were sown on December 14, 2018 and May 15, 2020, transplanted in blocks on January 3, 2019 and May 29, 2020, and finally transplanted in the greenhouse on January 31, 2019 and June 18, 2020, respectively. Mature green, breaker, turning, ripe and overripe fruits were harvested. The greenhouse was located in Horst (Netherlands). No cluster pruning was applied in this experiment.

The growth conditions were comparable to commercial tomato growing conditions in the Netherlands. The nutrient solutions provided had an electrical conductivity of 2,2 mS/cm. Immediately after harvesting, the fruits were transported to Wageningen where the samples were processed the same or next day. Per genotype/ripening stage 10 representative fruits were randomly selected. From each tomato, a wedge (top to button) with the size of 1/8 of the fruit was cut with a sharp knife and quickly frozen in liquid nitrogen in pools per genotype. The frozen wedges were ground into a fine powder per genotype using a liquid nitrogen-cooled grinder (IKA).

2.2. GABA and glutamic acid measurements

The levels of GABA and glutamic acid present in the tomato and potato samples were quantified using gas chromatography coupled to mass spectrometry (GCMS). The extraction and analysis procedures were mainly according to the protocol described previously (Carreno--Quintero et al., 2012). Briefly, 100 mg (< 2.5% deviation) of the frozen ground tomato or potato powder was weighed into a 2 mL Eppendorf tube. The powders were extracted with 1400 μL of a 90% (v:v) methanol/water solution containing 15 μg/mL of GABA-d6 and 50 μg/mL of L-glutamic acid-d5 (both from Merck-Sigma, Zwijndrecht, The Netherlands) as deuterium-labelled internal standards. After 10-minutes of sonication followed by 10-minutes of centrifugation, 500 μL of the clear supernatant was taken and mixed with 375 μL of chloroform and 750 μL of water. After a new centrifugation step, 50 μL aliquots of the upper (polar) phase were transferred into a glass insert placed in an open 2 mL GC vial. Extracts were dried overnight (16 h) by vacuum centrifugation (Savant®, SPD121P, Thermo Scientific, Breda, The Netherlands) at room temperature, and the vials were subsequently closed under an argon atmosphere with magnetic crimp caps. Prior to their analysis, dried extracts were derivatized online using a TriPlusRSH autosampling/injection robot (Thermo Scientific), which was programmed to firstly add 17.5 μL of O-methylhydroxylamine hydrochloride (20 mg/mL pyridine) (both from Merck-Sigma, Zwijndrecht, The Netherlands), then incubated for 30 min at 40 °C with agitation, and finally derivatized the compounds with 17.5 μL of N-methyl--*N*-trimethylsilyltrifluoroacetamide (MSTFA))(Merck-Sigma, Zwijndrecht, The Netherlands), for 60 min at 40 °C. After derivatization, compounds were separated on a Trace 1300 gas chromatograph system (Thermo Scientific). Of each sample 1 μL was injected onto a PTV injector (70 °C). By using a split flow of 19 mL/min samples were introduced onto a VF-5 ms capillary column + 10 m guard column (30 m × 0.25 mm × 0.25 μm, Agilent Technologies) for chromatographic separation. Helium (5.0) was used as carrier gas at a constant column flow rate of 1 mL/min. The temperature program of the GC oven started at 70 °C (2 min hold) and rose with 10 °C/min to 310 °C (with 10 min hold). Separated compounds were ionized by electron impact at 70 eV and mass spectra were acquired at full scan mode with a *m/z* range of 50–600 at an ion source temperature of 290 °C using a TSQ8000 DUO-series triple quadrupole mass spectrometer (Thermo Scientific). A dilution series of 0.1–500 μg/mL of both GABA and glutamic acid was used to quantify these compounds in the samples, while correcting for the recovery of their deuterated standards. Representative chromatograms are presented in supplementary material 1.

In view of the large numbers of samples, we chose for repeated extraction and analysis of a pool of powders from randomly chosen potato or tomato genotypes, rather than analyzing each individual powder in two or more technical replicates. This pool of powders was extracted 11 times in the same manner as the individual powders and analysed by GCMS before, after and throughout the series of the real samples. Based on these quality control samples (QCs), we were able to calculate the overall analytical variation, which was 5.8% for GABA and 16.2% for glutamic acid.

2.3. Domestic cooking methods

Single batches of potato tubers (cv. 'Agria', provided by Agrico Research) and tomato fruits (cv. 'Trevine', provided by Nunhems) were prepared with different domestic cooking techniques (Tables 1 and 2) to assess the effects on GABA and glutamic acid content. A laboratory sample consisted of 10 randomly selected tubers or fruits. All operations using raw samples were performed swiftly in order to prevent any degradation of the sample. Each cooking method was performed in triplicate on different days. After preparation and cooking, samples were immediately frozen in liquid nitrogen and stored at − 80 °C until analysis. Samples were ground to a fine powder using a liquid nitrogen-cooled grinder (IKA). Analysis of GABA and glutamic acid content was performed as described in Section 2.2. In order to correct GABA and glutamic acid levels for potential differences between preparation techniques due to differential moisture losses, the pre- and post-treatment dry matter content was determined from their ground powders, according to the official method recommended by the AOAC (Animal Feed—General., 2023).

Table 1
Preparation methods before cooking and cooking conditions for each cooking method applied to potato.

Cooking technique	Preparation	Cooking conditions
Raw	Peeled	-
Boiling	Peeled, 2 cm thick slices	100 °C for 10 min in water with a ratio of 1:3 (potato:water, v-v)
Frying	Peeled, 1 cm × 1 cm x length potato (French fry shaped)	6 min at 150 °C in sunflower oil, cooled, for 4 min at 175 °C in sunflower oil
Steaming	Peeled, 2 cm thick slices	Steamed for 20 min in a steam oven (RATIONAL SelfCookingCenter)
Baking	Peeled, 1 cm thick slices	Baked in a hot-air oven (RATIONAL SelfCookingCenter) for 15 min at 200 °C with 2 tbsp of oil
Open pan frying	Peeled, 5 mm thick slices	Baked on one side for 5 min and for 2.5 min on the other side at medium-high temperature in 2 tbsp. oil
Microwave cooking	Peeled, 2 cm thick slices	900 W for 11 min in microwave (Panasonic Pro II Type NE-1880) with 2 tbsp of water

Table 2
Preparation method before cooking and cooking conditions for each cooking method applied to tomato.

Cooking technique	Preparation	Cooking conditions
Raw	Whole fruits	-
Boiling	Whole fruits	15 min at 100 °C in water (tomatoes were submerged in water)
Baking	Whole fruits	Baked in a hot-air oven (RATIONAL SelfCookingCenter) for 15 min at 200 °C

2.4. Data analysis

Statistical analyses were performed using the R environment for statistical computing version 4.0.2 (https://www.R-project.org) and IBM SPSS Statistics version 25. To assess significant differences between market categories, an ANOVA was performed with a Tukey post hoc adjustment for multiple comparisons. An independent samples t-test was performed to assess differences between different cooking techniques and differences between peel and flesh. An ANOVA and ANCOVA with Bonferroni correction was used to assess differences in GABA and glutamic acid content over different ripening stages with genotype as a covariate. The correlations between compound levels over different years and between GABA and glutamic acid across samples were assessed using Pearson's correlation.

3. Results

3.1. Variation in GABA and glutamic acid levels across tomato and potato genotypes

3.1.1. Tomato

Contents of GABA and glutamic acid were determined for 98 different tomato genotypes and results are shown in Figs. 1 and 2, respectively. Categories were either cherry, cluster, heirloom, or snack

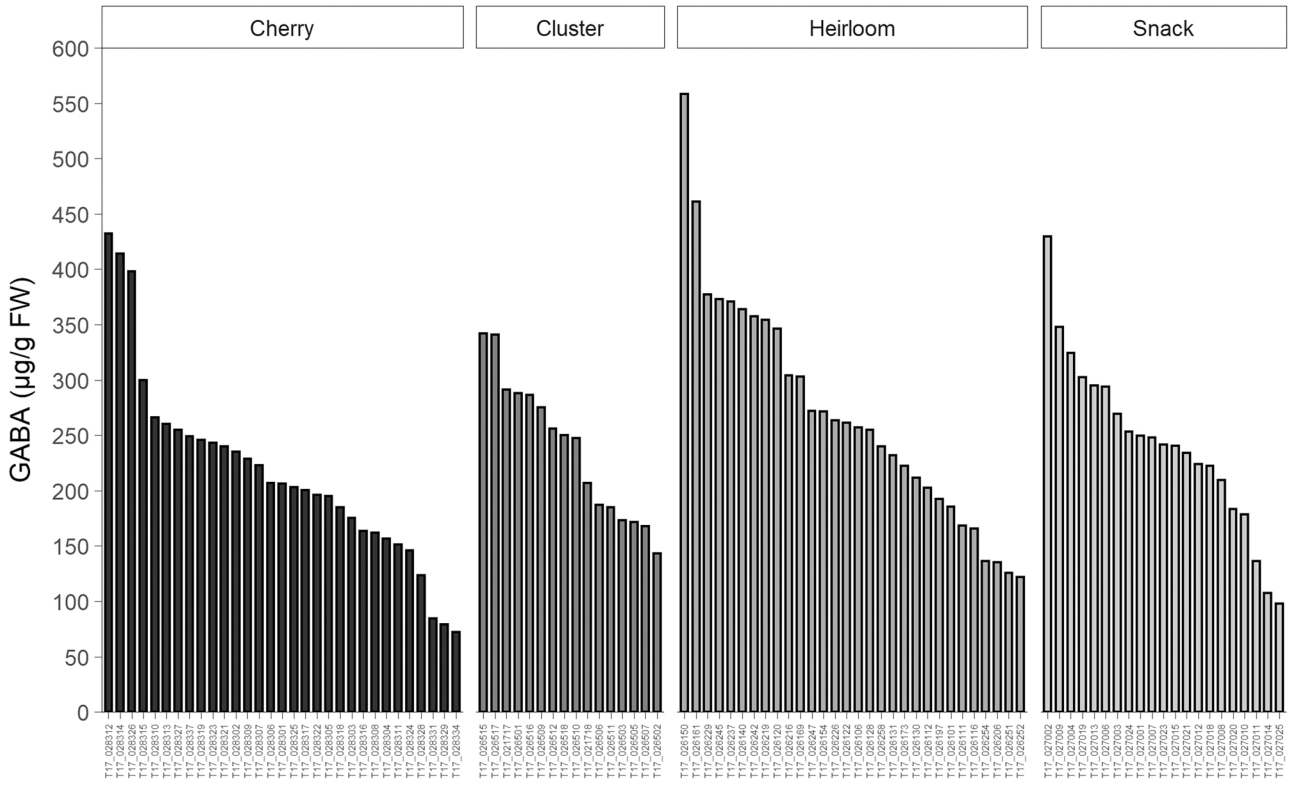

Fig. 1. GABA content per gram fresh weight of different tomato genotypes divided in cherry, cluster, heirloom and snack tomatoes. Genotypes are ordered from high to low GABA levels per fruit type. Each data point represents a pooled sample of 10 tomato fruits.

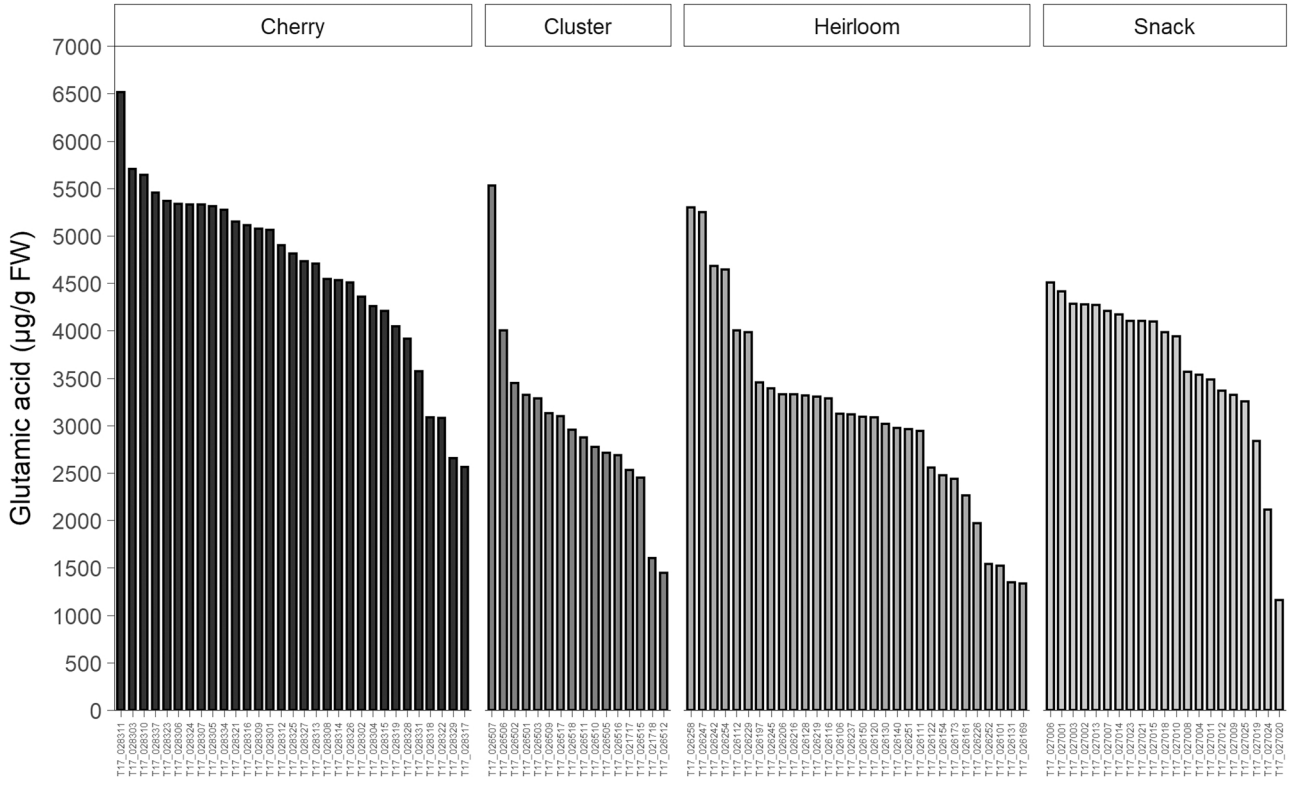

Fig. 2. Glutamic acid content per gram fresh weight of different tomato genotypes divided in cherry, cluster, heirloom and snack tomatoes. Genotypes are ordered from high to low glutamate levels per fruit type. Each data point represents a pooled sample of 10 tomato fruits.

tomatoes. On average, the tomato genotypes contained 242 ± 89 μg/g FW GABA, in a range from 72 to 558 μg/g FW. This corresponds with an 8-fold difference between the highest (found in a heirloom tomato fruit) and lowest (found in a cherry tomato fruit) accumulators of GABA. A one-way ANOVA revealed that there was no statistically significant difference in GABA content between the 4 market categories ($F(94, 3) = 1.92$, $p = 0.13$).

For glutamic acid, the average content was 3694 ± 1156 μg/g FW in a range from 1160 to 6513 μg/g FW. This resulted in a 6-fold difference between the highest (found in a cherry tomato fruit) and lowest (found in an heirloom tomato fruit) accumulators of glutamic acid. In this case, a one-way ANOVA showed that there was a statistically significant difference in glutamic acid content between the market categories ($F(94, 3) = 17.70$, $p = <0.001$). Tukey's HSD Test for multiple comparisons showed that the glutamic acid content of the cherry tomatoes (4649 ± 925 μg/g FW) was significantly higher than in the other market categories. No correlation was found between the GABA and glutamic acid content across the tomato fruits analyzed (Supplementary material 2).

3.1.2. Potato

The GABA and glutamic acid content was measured in 72 different potato cultivars and breeding clones. These represent a wide variety of market categories such as fresh retail category (e.g. Rosagold, Loreley), processing for fries (e.g. Fontane, Markies), for crisps (e.g. Hermes, Snowden), for starch extraction (Kuras), diploid cultivar group Phureja (Mayan Twilight, Papapura), Dutch germplasm (Armada, Artemis), Polish germplasm (Bzura, Strobrawa), UK germplasm (Maris Peer), German germplasm (Jelly, Regina) and USA germplasm (Premier Russet, Dakota Pearl). On average, these potato tubers contained 321 ± 132 μg/g FW GABA in a range from 68 to 759 μg/g FW (Fig. 3), while for glutamic acid the average was 571 ± 97 μg/g FW in a range from 409 to 874 μg/g FW (Fig. 4). Therefore, an 11-fold variation was observed for GABA and a 2-fold variation for glutamic acid. The cv. 'Riviera' stood out with a 33% higher GABA content than all other cultivars and breeding clones. Like in tomato fruit, no correlation was found between the GABA and glutamic acid content in potato tubers (Supplementary material 3).

3.2. The variation in effect of harvest year on GABA levels in tomato and potato and fruit maturation on GABA levels in tomato

3.2.1. Tomato

As differences in GABA content were observed in ripe tomato fruits, we analyzed a second series of genotypes that were harvested the same year (either 2018/2019 or 2020) but at different stages of their ripening. We aimed to determine what the relevance is of harvesting moment for the GABA content. For all genotypes, a decrease in GABA and an increase in glutamic acid was observed during fruit ripening (Fig. 5). On average the GABA content is significantly higher in green tomatoes as compared to all other stages and the glutamic acid content is significantly higher in overripe tomatoes as compared to the green and breaker stages. Genotype was only of significant influence on the relationship between ripening stage and GABA content ($p = < 0.001$) and not on the relationship between ripening stage and glutamic acid content ($p = 0.08$). Proportionally, the difference in GABA content between ripe and green tomato fruits is smallest in the Madara cultivar, while the difference in glutamic acid content is largest in this cultivar. The cv. 'Madara' also had an exceptionally high GABA level of 1122 ± 104 μg/g FW in its ripe fruits. We subsequently investigated the reproducibility of this relatively high GABA content in Madara, over different harvest years (Fig. 6). A one-way ANOVA showed that GABA content was not different between the different years ($F(25, 1) = 0.53$, $p = 0.47$), suggesting a constitutive relatively high level in 'Madara'.

3.2.2. Potato

We also assessed the reproducibility of the GABA content in potato genotypes across years. In a selection of 22 genotypes from the original screening in 2018 (Figs. 3 and 4), the GABA content was again

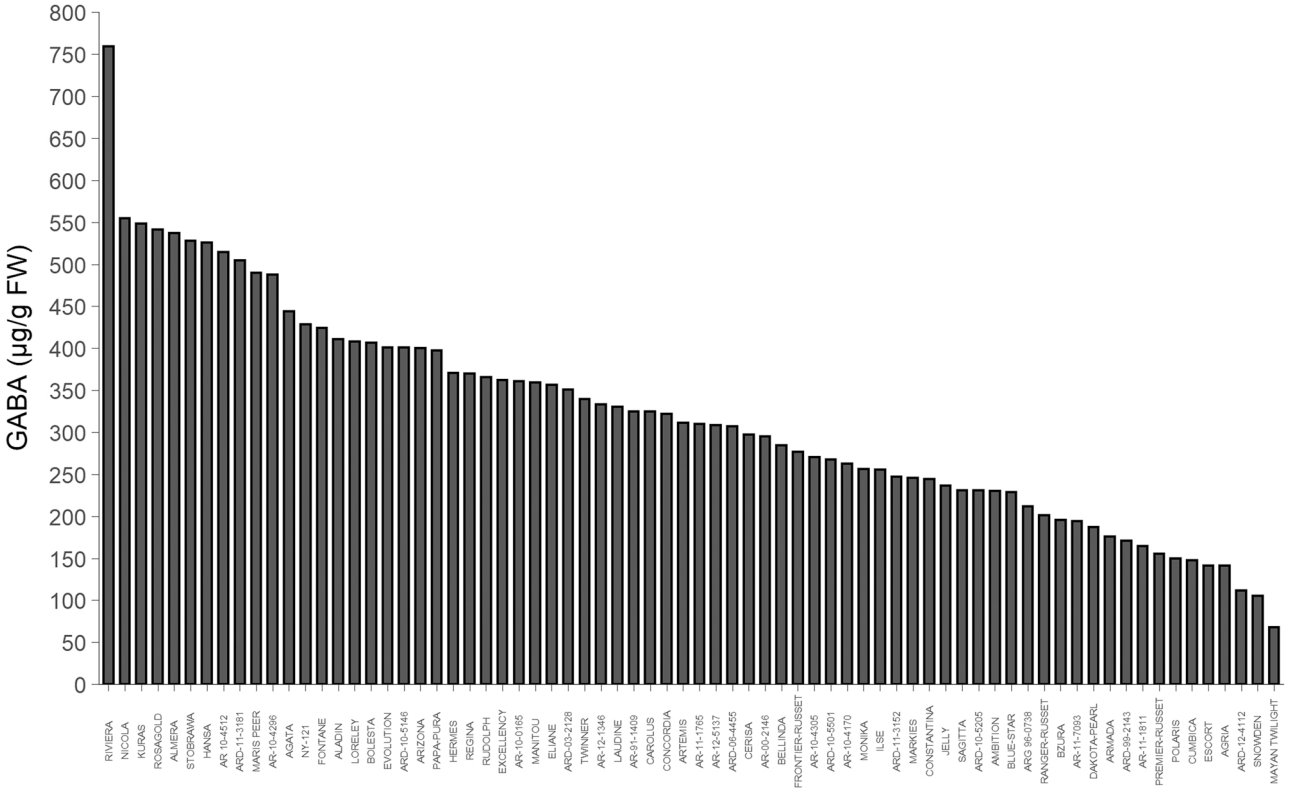

Fig. 3. GABA content per gram fresh weight of different potato genotypes. Each data point represents a pooled sample of 10 potato tubers.

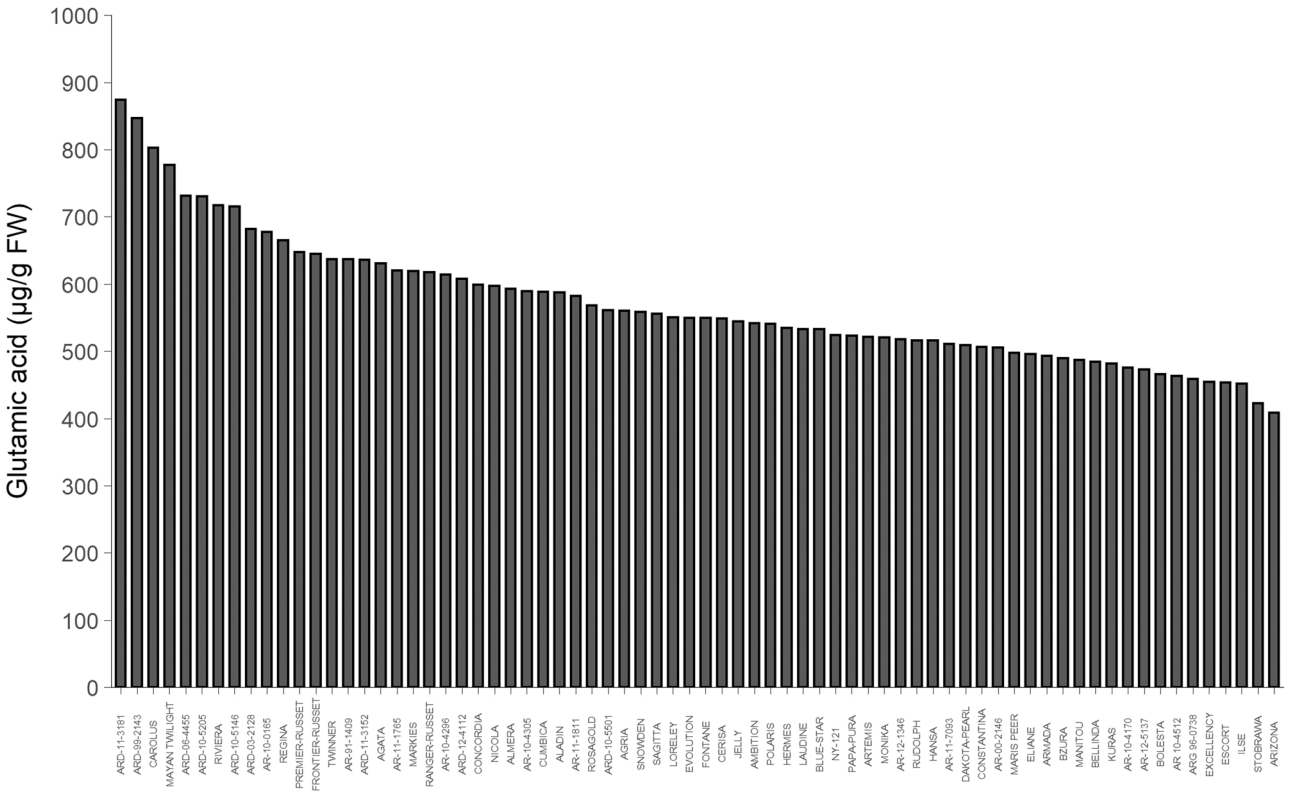

Fig. 4. Glutamic acid content per gram fresh weight of different potato genotypes. Each data point represents a pooled sample of 10 potato tubers.

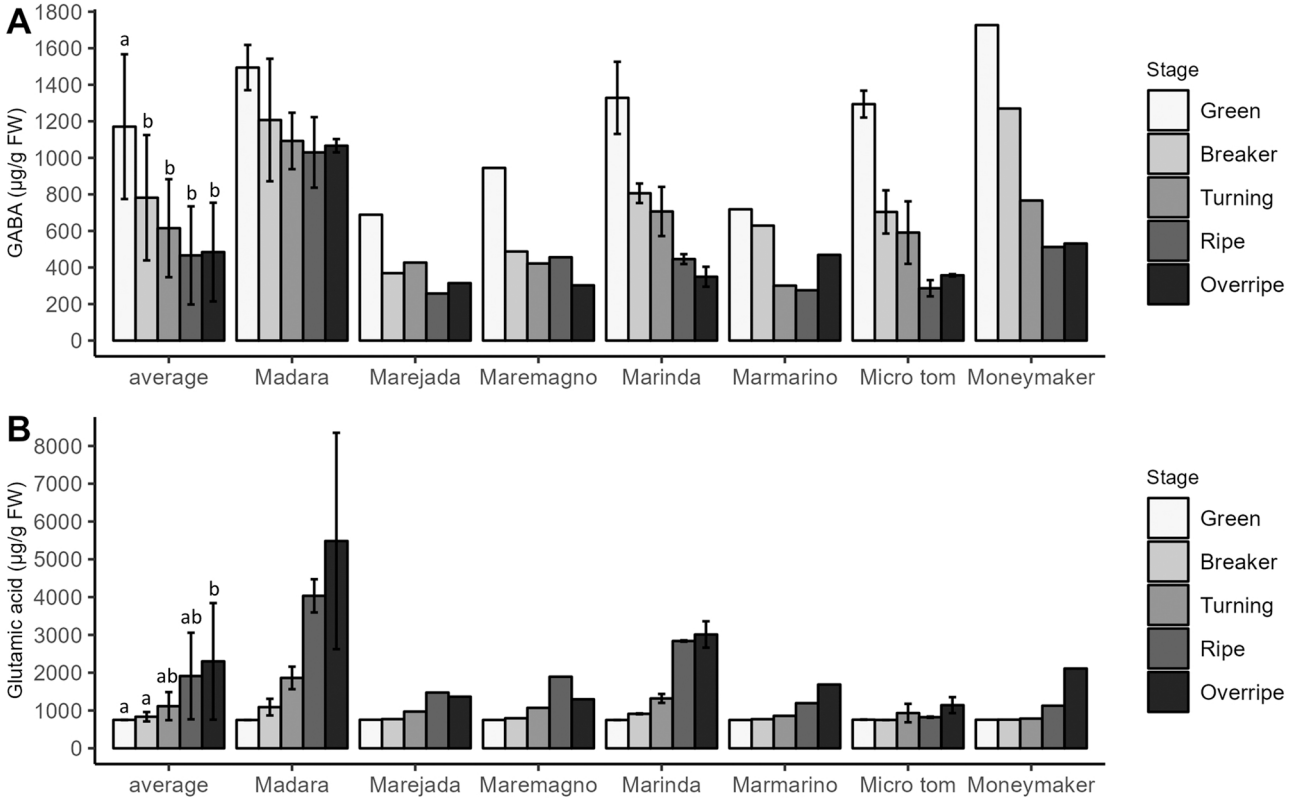

Fig. 5. A) GABA and B) glutamic acid content in 7 different tomato genotypes, over different stages of ripening(Green, Breaker, Turning, Ripe and Overripe). Each data point represents a pooled sample of 10 tomato fruits. A second harvest (same year, different dates) of cv 'Madara', 'Marinda' and 'Micro tom' was analyzed to assess batch variation in GABA and glutamic acid levels. For these 3 cultivars the data is presented as mean ± SD of the two replicates. The barplot also shows the overall average of all 7 genotypes with letters indicating significant differences between ripening stages.

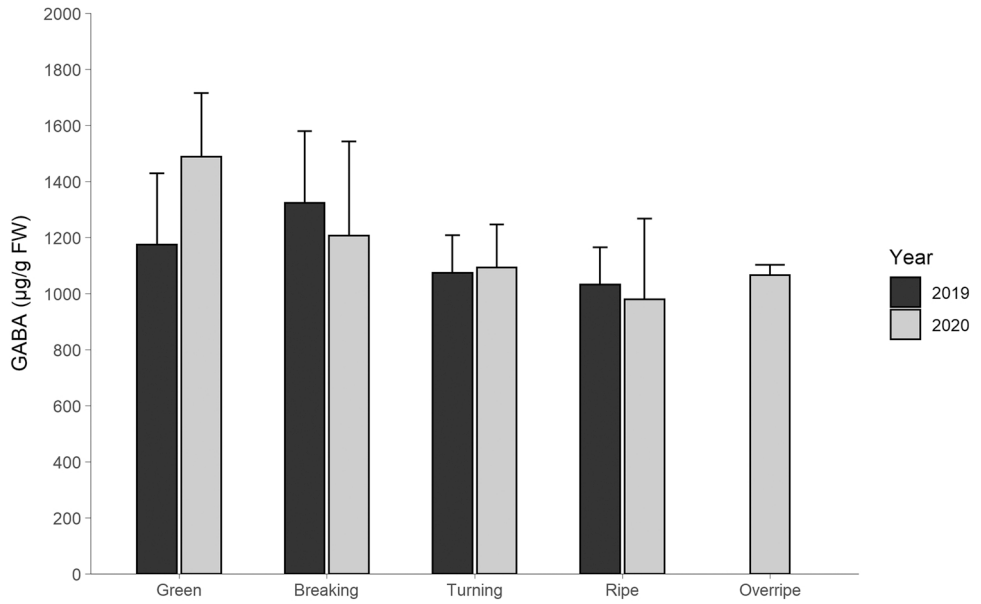

Fig. 6. GABA content measured in cv. 'Madara' over different stages of ripening in 2019 and 2020. Data are presented as mean and standard deviation. Replications represent different harvesting dates, on each date a pooled sample of 10 tomato fruits was sampled per ripening stage. n = 3 in 2019, n = 3 for the green and ripe stage in 2020 and n = 2 for the other stages in 2020.

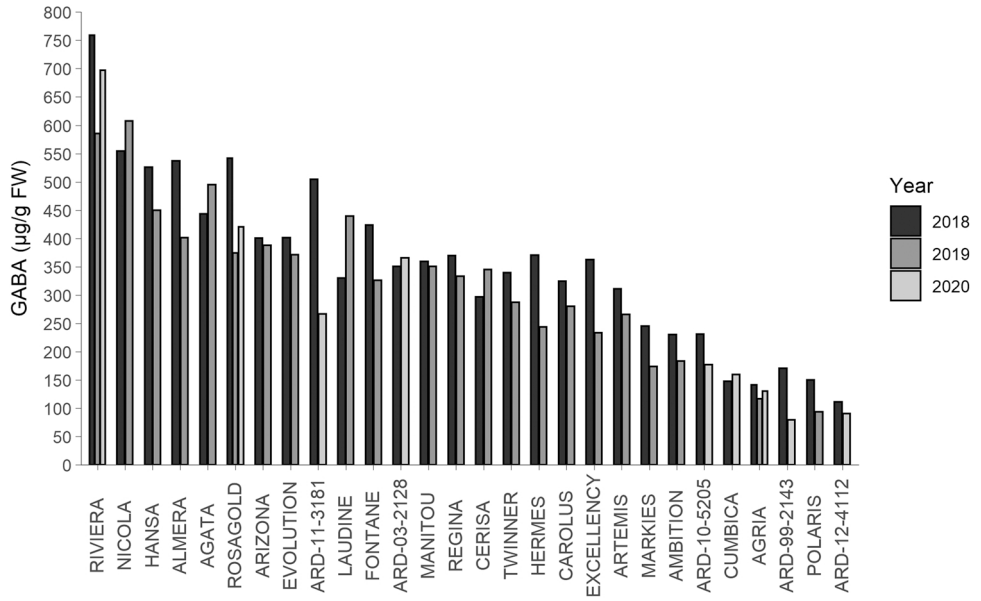

Fig. 7. Reproducibility of GABA content per gram fresh weight for potato genotypes grown in 2019 (n = 22) and/or 2020 (n = 9) as compared to tubers from 2018. Each value represents a pool of 10 potato tubers.

determined in tubers from the harvest in 2019 (Fig. 7) and for an additional 9 potato genotypes the GABA content was measured in tubers from the harvest in 2020. The tuber GABA content in 2019 and 2018 was highly correlated across similar genotypes (r = 0.87; 95% CI 0.70, 0.94; $P < 0.001$); also the levels obtained from harvest 2020 were highly correlated with those from harvest 2018 (r = 0.94; 95% CI 0.72, 0.99; $P < 0.001$).

3.3. *Effects of domestic cooking methods on GABA and glutamic acid levels*

Preparation of tomatoes and particularly potatoes frequently involves heat and other food-handling activities before their consumption. These cooking methods potentially affect the levels of GABA and glutamic acid in the consumed food products. To determine their impact, frequently employed potato and tomato cooking methods were applied and assessed for their effects on GABA and glutamic acid content. In these experiments, the compound levels were calculated on a dry weight basis, to account for potential differential water loss during product preparation. Table 3 shows the GABA and glutamic acid content per gram dry weight expressed as a percentage of the initial raw product. Dry weight measurements and GABA and glutamic acid content per gram of fresh weight are given in supplementary materials 4 and 5. As indicated in Table 3, in potato, most cooking methods resulted in considerable (12–52%) loss of glutamic acid. Also in tomato, boiling resulted in a significant (35%) loss of glutamic acid. Glutamic acid losses after baking tomatoes were more variable between replicate experiments (possibly due to differences in bursting of fruits). In potato, GABA

Table 3
Effects of different cooking methods on GABA and glutamic acid content based on dry weight and expressed as percentage of the raw sample. Cooking methods were replicated on 3 different days, each time a pooled sample from 10 fruits or tubers was taken.

Method	GABA[1] (% of raw)	Glutamic acid[1] (% of raw)
Potato		
Raw	100 (7.0)	100 (2.6)
Boiled	74.5 (19.2)·	88.5 (6.7)·
Fried	82.9 (2.1)*	47.8 (2.1)* **
Steamed	81.3 (8.8)*	88.2 (5.8)*
Baked	98.8 (7.3)	72.0 (6.7)* *
Pan-fried	84.1 (26.4)	71.9 (19.6)·
Microwaved	81.7 (3.6)*	77.1 (7.8)* *
Tomato		
Raw	100 (20.4)	100 (16.6)
Boiled	141.5 (18.7)·	64.7 (9.2)*
Baked	99.2 (10.9)	53.3 (25.5)·

1 Data is presented as mean (SD) percentage of the raw potato or tomato sample. Significant and trend differences are shown as compared to the raw potato or tomato sample, as assessed with an independent samples t-test. ·: $P < 0.10$; * : $P < 0.05$; * *: $P < 0.01$; * ** : $P < 0.001$.

levels were also significantly reduced after frying, steaming and microwaving, although to a lesser extent than glutamic acid (17–19%). Boiling of potatoes also tended to reduce their GABA content, while no effect was observed with baking and pan-frying. In contrast to its decreasing effect in potato, boiling tended to increase the GABA content in tomatoes (42%, $P = 0.06$), while baking the tomatoes did not alter their content.

To study these effects of various domestic cooking methods, peeled potatoes were used. In order to determine the distribution of both GABA and glutamic acid between peel and remaining tuber (flesh), we separately analyzed peel and flesh tissue from tubers of 10 different potato genotypes for both GABA and glutamic acid content (Fig. 8). The difference in GABA content between the peel and flesh varied between genotypes, while no significant difference was observed on average. In contrast, the glutamic acid content was consistently and significantly higher in the peel than in the flesh.

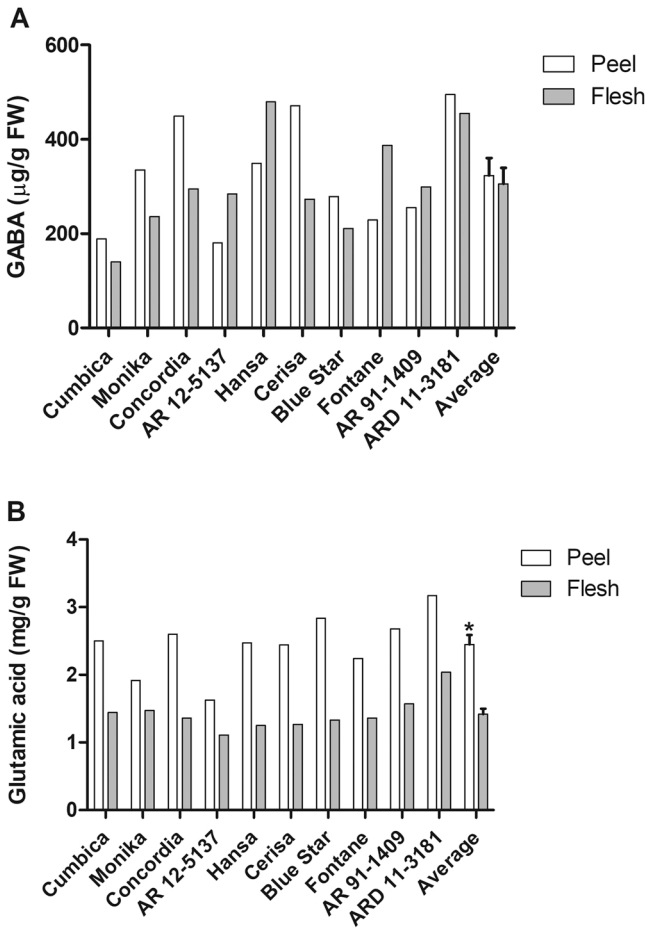

Fig. 8. Comparison of A) GABA and B) glutamic acid content between peel and flesh. Values are shown from pooled samples of 10 tubers. As a last bar, averages of the peel and flesh of the presented genotypes are shown as mean and SD. * indicates a significant difference.

4. Discussion

In search of tomato and potato varieties with a relatively high GABA content, a large selection of 98 tomato and 72 potato genotypes was analyzed. The concentration of both GABA and its precursor glutamic acid was assessed and substantial natural variation was found to be present in GABA (11- and 14-fold resp.) and glutamic acid (2- and 6-fold resp.) levels in both potato and tomato. Although glutamic acid is the direct precursor in the biosynthesis of GABA, their levels do not correlate across genotypes analyzed. In ripening tomato fruit, the GABA content generally decreases while glutamic acid content increases. Interestingly, the cultivar containing the highest GABA level in ripe fruits, i.e. cv. 'Madara', also retained the most GABA during ripening. In addition, the GABA content of both tomato (only assessed for Madara) and potato (various genotypes) was found to be reproducible across different growing seasons. Specific genotypes of tomato (e.g. Madara with ~1000 ug/g) and potato (e.g. Riviera with ~700 ug/g) could therefore be a relevant source of GABA in the human diet, especially because GABA was found to be largely tolerant to diverse commonly used domestic cooking methods (at most 25% decrease). In contrast, the glutamic acid content was found to decrease more as a result of domestic cooking.

The genetic variation in GABA content between tomato genotypes (including cv. 'Madara') as reported in the current paper, is similar to that previously found in other studies. A range from approximately 90 to 1000 µg/g FW was observed in a similarly large screening of tomato genotypes (Saito et al., 2008). The GABA content in potato has not been screened extensively before. Nakamura et. al (2006) found a GABA content ranging from 239 to 819 µg/g FW in 22 potato genotypes (Nakamura et al., 2006). The range observed is larger and also includes genotypes with a lower GABA content, most likely because more genotypes were included. The lowest level observed in potato was 68 µg/g FW.

Literature suggests that the levels of GABA and its biosynthetic precursor glutamic acid are linked (Akihiro et al., 2008), although this hypothesis has not been assessed across a wide range of genotypes. In the current study, no clear correlation between GABA and glutamic acid levels across genotypes was found, neither in mature tomato nor in potato. This result suggests that breeding towards a higher GABA content does not automatically lead to changes in glutamic acid content. This is especially important for tomato fruits, in which free glutamic acid is one of the main compounds responsible for its "umami" taste (Tommonaro et al., 2021).

In this study, the potential causes of the observed natural variation in GABA content was not investigated. GABA is primarily produced by glutamic acid decarboxylase (GAD) from glutamic acid and catabolized by GABA transaminase (GABA-T) into succinate (Koike et al., 2013). GABA content is, therefore, likely determined by the activity of these two enzymes. Indeed, targeted mutagenesis in the GAD gene effectively increases the GABA content in tomato, reaching levels up to 1250 µg/g FW (Nonaka et al., 2017). In the current study, cv. 'Madara' appeared to be able to accumulate levels up to ~1000 µg/g FW, which is in the same range as the GAD-gene-edited tomato. However, for reliable

quantitative comparisons it will be necessary to grow these gene-edited and natural genotypes together under the same conditions, harvest them at the same ripening stage and analyze them with the same analytical methods.

In general, a decrease in GABA content and an increase in glutamic acid content was observed during the tomato fruit ripening process. Previously, it was shown that the GABA content of fruits from cv. 'Micro Tom' decreases about 6-fold from the mature green to the red ripe stage of their ripening (Akihiro et al., 2008). A similar 5-fold decrease in this cv. 'Micro Tom' was observed. A decrease in the GABA content of the high-GABA cv. Madara was also observed, but this decrease was relatively small (1.5-fold) compared to that in both Micro Tom and to other cultivars. Similar to the GABA-rich *DG03–9* variety described by Saito et al. (2008), Madara also seems to lose less GABA during ripening (Akihiro et al., 2008; Saito et al., 2008). Such relatively low loss is possibly due to a differential ripening-related regulation of either GAD activity or GABA-T activity, or both, as compared to other genotypes (Takayama and Ezura, 2015). Other than the *DG03–9* variety, Madara also seems to accumulate glutamic acid more and earlier in the ripening process compared to other genotypes. Future studies focusing on this differential regulation may provide more insight into the mechanism(s) behind a relatively high GABA level in ripe tomato fruit. Crossing these two high-GABA varieties might help to explore any genetic synergy and to produce offspring with even higher GABA levels.

The GABA content was reproducible across different growing seasons, as was observed for tomato cv 'Madara' and a range of potato genotypes. In contrast, Saito et al. (2008) showed poor reproducibility of tomato fruit GABA content over years (Saito et al., 2008). However, in contrast to the current study, the tomato plants of Saito et al. (2008) were grown in the field. Since GABA content can be influenced by environmental stressors (Bown and Shelp, 2016; Kinnersley and Turano, 2010), it is well possible that differences in growth conditions between years could have caused this discrepancy. The standardized greenhouse growth conditions for tomato, as used in the current study, may better ensure a reproducible GABA content (only 9.7% difference within genotype between years on average) than field conditions.

Tomatoes are often processed before their consumption and potatoes are never eaten raw. Therefore, the effect of popular domestic cooking methods on the GABA and glutamic acid levels in tomato and potato was also investigated. Consistent with the literature, GABA appeared to be relatively more resistant to most of the preparation processes than glutamic acid (Li et al., 2017; Ma et al., 2022). Frying, steaming, and microwaving potatoes decreased the GABA content by ~20% in potatoes, while also boiling tended to decrease GABA. While in tomato no decrease in GABA was observed by any treatment, glutamic acid was significantly lowered by boiling and baking. In the experiments described in this manuscript, the tomatoes were boiled as whole fruits including peel. Removing the peel may potentially lead to more losses, as both GABA and glutamic acid are potentially able to dissolve into the boiling water. For potato the peel was removed from the tubers before cooking. Preparing the potatoes with the peel still present thus could possibly have prevented the observed GABA losses. Interestingly, boiling tended to increase the GABA content in tomatoes by approximately 40%, whereas the glutamic acid content was decreased by 35%. GABA is known to accumulate in response to several abiotic stress conditions like cold, heat, and salt (Kinnersley and Turano, 2010). Prolonged heat-drying of green soybeans up to a temperature of 40 degrees increased GABA content by a factor 5 for example (Takahashi et al., 2013). Thus, a possible explanation for the observed increased GABA level in boiled tomatoes is an (temporarily) ongoing or even increased GAD activity upon heating. Further research on the effect of moderate heat treatment on GABA accumulation might lead to a recommendation of slow cooking as an appropriate processing method to additionally increase GABA in intact tomatoes and possibly also in intact, non-peeled potatoes.

The presented effects of domestic cooking were calculated on dry weight, to take into account the difference in water losses between cooking methods. From a nutritional perspective, the GABA and glutamic acid content should also be considered per gram of fresh weight and portion size. For example, frying results in substantial water loss, which concentrates the GABA and glutamic acid levels. This leads to a high GABA content, expressed per gram of food product in fried potatoes (165 μg/g FW in fried potato versus 103 μg/g FW in raw potato, Supplementary material 5). On the other hand, for glutamic acid, this concentrating effect of frying is not able to compensate for the frying-induced loss of glutamic acid (0.9 mg/g FW in fried potato versus 0.97 mg/g FW in raw potato, Supplementary material 5).

It was chosen to investigate the effect of cooking on peeled potatoes, but they can also be prepared unpeeled. For potato consistently higher glutamic acid levels in tuber peel as compared to flesh was shown across multiple potato genotypes, while for GABA no consistent differences between peel and flesh were found. Talley et al. (1983) found that, in general, the potato peel was higher in nitrogen and free amino acid contents than the potato flesh, although the exception in their samples was for glutamic acid and GABA (Talley et al., 1983).

5. Conclusion

Tomato and potato genotypes relatively high in GABA, could be a good source of GABA as part of a natural GABA-rich diet. Other food sources may contain more GABA but are rarely eaten in larger quantities than potato and tomato. Melons for example can contain even up to 3000 μg/g FW GABA, but the average intake of melons is low in the EU (ranging from 2.3 g per day in the Netherlands to 14 g a day in Italy) (Mertena et al., 2011; Toyoizumi et al., 2019; van Rossum et al., 2020). This means that such high GABA-containing melons only contribute about 7–40 mg to the daily GABA intake. Based on the EU-average intake of 233 g potato and 92 g tomato per day and assuming the average genotype GABA content of raw potato (321 μg/g FW) and tomato (242 μg/g FW), people in the EU currently receive 75 mg GABA/day from potato and 22 mg/day from tomato consumption, which translates into 97 mg GABA/day total excluding losses or gains from cooking (Directorate-General for Agriculture and Rural Development. (2021); Wijesinha-Bettoni and Mouillé, 2019). If the highest GABA potato (cv. 'Riviera') and tomato (cv. 'Madara') cultivars would be consumed, this intake could increase to 173 mg per day from potato and 90 mg per day from tomato, i.e. 263 mg GABA/day in total. With a focused effort on breeding, growth conditions and food treatments this intake could likely be even higher. However, it is yet unclear what would be an effective GABA dose to exert significant health effects in humans, because the human intervention studies that were done so far did not have proper controls to relate the observed effects to GABA alone (Inoue et al., 2003; Shimada et al., 2009; Tanaka et al., 2009). We recently showed that in humans GABA is readily taken up into the blood stream upon consuming pureed tomatoes (Bie et al., 2022). In addition, the GABA content of both tomato and potato is reproducible across different years of harvest and seems to be quite stable during further preparation like cooking. In conclusion, high GABA-containing potatoes and tomatoes could be promising additions to a healthy diet.

CRediT authorship contribution statement

Tessa H. de Bie: Formal analysis, Investigation, Visualization, Writing – original draft. **Ric C.H. de Vos:** Conceptualization, Data curation, Investigation, Methodology, Supervision, Validation, Writing – review & editing. **Henriëtte D.L.M. van Eekelen:** Data curation, Investigation, Validation. **Frank F. Millenaar:** Resources, Writing – review & editing. **Cindy K.M. van de Wiel:** Resources, Methodology. **Josephus J.H.M. Allefs:** Resources, Writing – review & editing, **Michiel G.J. Balvers:** Supervision. **Renger F. Witkamp:** Funding acquisition, Supervision. **Maarten A. Jongsma:** Conceptualization, Funding acquisition, Supervision, Writing – review & editing.

Declaration of Competing Interest

No conflicts of interest.

Data Availability

Data will be made available on request.

Acknowledgments

Roland Mumm, Jeroen van Arkel and Bert Schipper from the Plant Metabolomics lab of Bioscience, WUR, are acknowledged for their help with cutting and grinding the fruits and with the analysis of GABA and glutamic acid of both the tomato and potato samples. Marit Hoppenreijs is acknowledged for her help in cooking the potatoes and tomatoes and analyzing the samples. Arnaud Bovy is acknowledged for providing access to the non-published data of a large genotype screen and providing us with seeds of the Madara variety that was highest in GABA.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jfca.2023.105416.

References

Akihiro, T., Koike, S., Tani, R., Tominaga, T., Watanabe, S., Iijima, Y., Aoki, K., Shibata, D., Ashihara, H., Matsukura, C., Akama, K., Fujimura, T., Ezura, H., 2008. Biochemical mechanism on GABA accumulation during fruit development in tomato. Plant Cell Physiol. 49, 1378–1389. https://doi.org/10.1093/pcp/pcn113.

Animal Feed—General, 2023. Official Methods of Analysis of AOAC INTERNATIONAL. https://doi.org/10.1093/9780197610145.003.037.

Baldina, S., Picarella, M.E., Troise, A.D., Pucci, A., Ruggieri, V., Ferracane, R., Barone, A., Fogliano, V., Mazzucato, A., 2016. Metabolite profiling of Italian tomato landraces with different fruit types. Front Plant Sci. 7, 664. https://doi.org/10.3389/fpls.2016.00664.

Bie, T.H. de, Balvers, M.G.J., Vos, R.C.H. de, Witkamp, R.F., Jongsma, M.A., 2022. The influence of a tomato food matrix on the bioavailability and plasma kinetics of oral gamma-aminobutyric acid (GABA) and its precursor glutamate in healthy men. Food Funct. 16, 8399–8410. https://doi.org/10.1039/D2FO01358D.

Bown, A.W., Shelp, B.J., 2016. Plant GABA: not just a metabolite. Trends Plant Sci. 21, 811–813. https://doi.org/10.1016/j.tplants.2016.08.001.

Brosnan, J.T., Brosnan, M.E., 2013. Glutamate: a truly functional amino acid. Amino Acids 45, 413–418. https://doi.org/10.1007/s00726-012-1280-4.

Carreno-Quintero, N., Acharjee, A., Maliepaard, C., Bachem, C.W.B., Mumm, R., Bouwmeester, H., Visser, R.G.F., Keurentjes, J.J.B., 2012. Untargeted metabolic quantitative trait loci analyses reveal a relationship between primary metabolism and potato tuber quality. Plant Physiol. 158, 1306–1318. https://doi.org/10.1104/pp.111.188441.

Directorate-General for Agriculture and Rural Development, 2021. Eu agricultural outlook for the agricultural markets and income 2021–2031, Agriculture and Rural Development. https://doi.org/10.2762/753688.

Dobson, G., Shepherd, T., Verrall, S.R., Conner, S., Mcnicol, J.W., Ramsay, G., Shepherd, L.V.T., Vies, H.V.D., Stewart, D., 2008. Phytochemical diversity in tubers of potato cultivars and landraces using a gc-ms metabolomics approach. J. Agric. Food Chem. 56, 10280–10291. https://doi.org/10.1021/jf801370b.

Fait, A., Fromm, H., Walter, D., Galili, G., Fernie, A.R., 2008. Highway or byway: the metabolic role of the GABA shunt in plants. Trends Plant Sci. 13, 14–19. https://doi.org/10.1016/J.TPLANTS.2007.10.005.

Forde, B.G., Lea, P.J., 2007. Glutamate in plants: metabolism, regulation, and signalling. J. Exp. Bot. Oxf. Acad. 2339–2358. https://doi.org/10.1093/jxb/erm121.

Gramazio, P., Takayama, M., Ezura, H., 2020. Challenges and prospects of new plant breeding techniques for GABA improvement in crops: tomato as an example. Front Plant Sci. 11, 1382. https://doi.org/10.3389/fpls.2020.577980.

Halford, N.G., Muttucumaru, N., Powers, S.J., Gillatt, P.N., Hartley, L., Elmore, J.S., Mottram, D.S., 2012. Concentrations of free amino acids and sugars in nine potato varieties: effects of storage and relationship with acrylamide formation. J. Agric. Food Chem. 60, 12044–12055. https://doi.org/10.1021/jf3037566.

Hayakawa, K., Kimura, M., Kasaha, K., Matsumoto, K., Sansawa, H., Yamori, Y., 2004. Effect of a γ-aminobutyric acid-enriched dairy product on the blood pressure of spontaneously hypertensive and normotensive Wistar–Kyoto rats. Br. J. Nutr. 92, 411. https://doi.org/10.1079/BJN20041221.

Herawati, H., Afifah, D.N., Kusumanigtyas, E., Usmiati, S., Soemantri, A.S., Miskiyah, Kamsiati, E., Bachtiar, M., 2021. Characterization of GABA (gamma-aminobutyric acid) levels some fermented food in Indonesia. IOP Conf. Ser. Earth Environ. Sci. 819, 012068 https://doi.org/10.1088/1755-1315/819/1/012068.

Inoue, K., Shirai, T., Ochiai, H., Kasao, M., Hayakawa, K., Kimura, M., Sansawa, H., 2003. Blood-pressure-lowering effect of a novel fermented milk containing γ-aminobutyric acid (GABA) in mild hypertensives. Eur. J. Clin. Nutr. 57, 490–495. https://doi.org/10.1038/sj.ejcn.1601555.

Kinnersley, A.M., Turano, F.J., 2010. Gamma aminobutyric acid (GABA) and plant responses to stress. CRC Crit. Rev. Plant Sci. 19, 479–509. https://doi.org/10.1080/07352680091139277.

Koike, S., Matsukura, C., Takayama, M., Asamizu, E., Ezura, H., 2013. Suppression of γ-Aminobutyric Acid (GABA) Transaminases Induces Prominent GABA Accumulation, Dwarfism and Infertility in the Tomato (Solanum lycopersicum L.). Plant Cell Physiol. 54, 793–807. https://doi.org/10.1093/PCP/PCT035.

Li, J., Zhang, Z., Liu, X., Wang, Y., Mao, F., Mao, J., Lu, X., Jiang, D., Wan, Y., Lv, J.-Y., Cao, G., Zhang, J., Zhao, N., Atkinson, M., Greiner, D.L., Prud'homme, G.J., Jiao, Z., Li, Y., Wang, Q., 2015. Study of GABA in healthy volunteers: pharmacokinetics and pharmacodynamics. Front Pharm. 6, 260. https://doi.org/10.3389/fphar.2015.00260.

Li, J.L., Tu, Z.C., Sha, X.M., Zhang, L., Lin, D.R., Zeng, K., Wang, H., Pang, J.J., Tang, P. P., 2017. Effect of frying on fatty acid profile, free amino acids and volatile compounds of grass carp (Ctenopharyngodon idellus) fillets. J. Food Process Preserv 41, e13088. https://doi.org/10.1111/JFPP.13088.

Ma, Y., Wang, A., Yang, M., Wang, S., Wang, L., Zhou, S., Blecker, C., 2022. Influences of cooking and storage on γ-aminobutyric acid (GABA) content and distribution in mung bean and its noodle products. LWT 154, 112783. https://doi.org/10.1016/J.LWT.2021.112783.

Mertena, C., Ferrari, P., Bakker, M., Boss, A., Hearty, A., Leclercq, C., Lindtner, O., Tlustos, C., Verger, P., Volatier, J.L., Arcella, D., 2011. Methodological characteristics of the national dietary surveys carried out in the European Union as included in the European Food Safety Authority (EFSA) comprehensive european food consumption database. Food Addit. Contam.: Part A 28, 975–995. https://doi.org/10.1080/19440049.2011.576440.

Nakamura, K., Nara, K., Noguchi, T., Oshiro, T., Koga, H., 2006. Contents of gamma-aminobutyric acid (GABA) in potatoes and processed potato products. J. Jpn. Soc. Food Sci. Technol. 53, 514–517.

Nonaka, S., Arai, C., Takayama, M., Matsukura, C., Ezura, H., 2017. Efficient increase of Γ-aminobutyric acid (GABA) content in tomato fruits by targeted mutagenesis. Sci. Rep. 7, 1–14. https://doi.org/10.1038/s41598-017-06400-y.

Pęksa, A., Miedzianka, J., Nemś, A., Rytel, E., 2021. The free-amino-acid content in six potatoes cultivars through storage. Molecules 26, 1322. https://doi.org/10.3390/molecules26051322.

Pratta, G., Zorzoli, R., Boggio, S.B., Picardi, L.A., Valle, E.M., 2004. Glutamine and glutamate levels and related metabolizing enzymes in tomato fruits with different shelf-life. Sci. Hortic. 100, 341–347. https://doi.org/10.1016/j.scienta.2003.08.004.

Ramesh, S.A., Tyerman, S.D., Gilliham, M., Xu, B., 2017. γ-Aminobutyric acid (GABA) signalling in plants. Cell. Mol. Life Sci. 74, 1577–1603. https://doi.org/10.1007/s00018-016-2415-7.

Redruello, B., Szwengiel, A., Ladero, V., Rio, B. del, Alvarez, M.A., 2020. Identification of technological/metabolic/environmental profiles of cheeses with high GABA contents. LWT 130, 109603. https://doi.org/10.1016/J.LWT.2020.109603.

Saito, T., Matsukura, C., Sugiyama, M., Watahiki, A., Ohshima, I., Iijima, Y., Konishi, C., Fujii, T., Inai, S., Fukuda, N., Nishimura, S., Ezura, H., 2008. Screening for γ-aminobutyric acid (GABA)-rich tomato varieties. J. Jpn. Soc. Hortic. Sci. 77, 242–250. https://doi.org/10.2503/jjshs1.77.242.

Scholz, S.S., Reichelt, M., Mekonnen, D.W., Ludewig, F., Mithöfer, A., 2015. Insect herbivory-elicited gaba accumulation in plants is a wound-induced, direct, systemic, and jasmonate-independent defense response. Front Plant Sci. 6, 1128. https://doi.org/10.3389/FPLS.2015.01128/BIBTEX.

Shimada, M., Hasegawa, T., Nishimura, C., Kan, H., Kanno, T., Nakamura, T., Matsubayashi, T., 2009. Anti-hypertensive effect of gamma-aminobutyric acid (GABA)-rich Chlorella on high-normal blood pressure and borderline hypertension in placebo-controlled double blind study. Clin. Exp. Hypertens. 31, 342–354. https://doi.org/10.1080/10641960902977908.

Skurray, G.R., Pucar, N., 1988. l-glutamic acid content of fresh and processed foods. Food Chem. 27, 177–180. https://doi.org/10.1016/0308-8146(88)90060-X.

Sorrequieta, A., Ferraro, G., Boggio, S.B., Valle, E.M., 2010. Free amino acid production during tomato fruit ripening: a focus on l-glutamate. Amino Acids 38, 1523–1532. https://doi.org/10.1007/s00726-009-0373-1.

Takahashi, Y., Sasanuma, T., Abe, T., 2013. Accumulation of gamma-aminobutyrate (GABA) caused by heat-drying and expression of related genes in immature vegetable soybean (edamame). Breed. Sci. 63, 205–210. https://doi.org/10.1270/JSBBS.63.205.

Takayama, M., Ezura, H., 2015. How and why does tomato accumulate a large amount of GABA in the fruit? Front Plant Sci. 6, 612. https://doi.org/10.3389/fpls.2015.00612.

Talley, E.A., Toma, R.B., Orr, P.H., 1983. Composition of raw and cooked potato peel and flesh: amino acid content. J. Food Sci. 48, 1360–1361. https://doi.org/10.1111/J.1365-2621.1983.TB09234.X.

Tanaka, H., Watanabe, K., Ma, M., Hirayama, M., Kobayashi, T., Oyama, H., Sakaguchi, Y., Kanda, M., Kodama, M., Aizawa, Y., 2009. The effects of gamma-aminobutyric acid, vinegar, and dried bonito on blood pressure in normotensive and mildly or moderately hypertensive volunteers. J. Clin. Biochem Nutr. 45, 93–100. https://doi.org/10.3164/jcbn.09-04.

Tian, J., Dang, H.N., Yong, J., Chui, W.-S., Dizon, M.P.G., Yaw, C.K.Y., Kaufman, D.L., 2011. Oral treatment with γ-aminobutyric acid improves glucose tolerance and insulin sensitivity by inhibiting inflammation in high fat diet-fed mice. PLoS One 6, e25338. https://doi.org/10.1371/journal.pone.0025338.

Tommonaro, G., Morelli, C.F., Rabuffetti, M., Nicolaus, B., De Prisco, R., Iodice, C., Speranza, G., 2021. Determination of flavor-potentiating compounds in different

Italian tomato varieties. J. Food Biochem 45, 13736. https://doi.org/10.1111/jfbc.13736.

Toyoizumi, T., Ohba, S., Fujii, K. suke, Ikegaya, A., Matsuura, H., Nakajima, T., 2019. Differential GABA concentration gradients are present in the edible parts of greenhouse melon (Cucumis melo L.) during all four seasonal croppings. Biosci. Biotechnol. Biochem 83, 330–338. https://doi.org/10.1080/09168451.2018.1537774.

Toyota, M., Spencer, D., Sawai-Toyota, S., Jiaqi, W., Zhang, T., Koo, A.J., Howe, G.A., Gilroy, S., 2018. Glutamate triggers long-distance, calcium-based plant defense signaling. Science (1979) 361, 1112–1115. https://doi.org/10.1126/science.aat7744.

Uri, C., Juhász, Z., Polgár, Z., Bánfalvi, Z., 2014. A GC-MS-based metabolomics study on the tubers of commercial potato cultivars upon storage. Food Chem. 159, 287–292. https://doi.org/10.1016/j.foodchem.2014.03.010.

van Rossum, C., Buurma-Rethans, E., Dinnissen, C., Beukers, M., Brants, H., Ocké, M., 2020. The diet of the Dutch: Results of the Dutch National Food Consumption Survey 2012–2016. RIVM letter report 2016–0082. https://doi.org/10.21945/RIVM-2020–0083.

Wijesinha-Bettoni, R., Mouillé, B., 2019. The contribution of potatoes to global food security, nutrition and healthy diets. Am. J. Potato Res. 2019 96 2 96, 139–149. https://doi.org/10.1007/S12230-018-09697-1.

Yang, Xiaozhe, Hu, W., Xiu, Z., Jiang, A., Yang, Xiangyan, Saren, G., Ji, Y., Guan, Y., Feng, K., 2020. Microbial community dynamics and metabolome changes during spontaneous fermentation of northeast sauerkraut from different households. Front Microbiol 11, 1878. https://doi.org/10.3389/FMICB.2020.01878.

Zushi, K., Matsuzoe, N., 2007. Salt stress-enhanced γ-aminobutyric acid (GABA) in tomato fruit. Acta Hortic. Int. Soc. Hortic. Sci. 431–435. https://doi.org/10.17660/ActaHortic.2007.761.59.