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Amino acids release from enriched bread with edible insect or pea protein during *in vitro* gastrointestinal digestion

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Abstract

The aim of this study was to investigate the amino acid (AA) release from breads enrichment with edible insects, *Alphitobius diaperinus* and *Tenebrio molitor* or pea protein during *in vitro* gastrointestinal digestion. Bread was enriched at 5 and 10% with insect flour or pea protein. Enriched and control breads were subjected to standardised static *in vitro* gastrointestinal digestion. The free AAs of breads before and after each phase of digestion (gastric, intestinal and at the end of digestion) were determined by HPLC. During digestion, the highest AA release from breads occurred in the intestinal phase. Using pea protein, *Alphitobius diaperinus,* and *Tenebrio molitor* powder at any level assayed presented a significantly higher value of total free AA than the control, accessible for body absorption. There is an effect of enrichment ingredient concentration (10 > 5%) in bread on total AA release after *in vitro* gastrointestinal digestion. Higher protein enrichment induced higher realease of AA during the digestion.

Keywords: edible insect, pea protein, amino acids release, bread

1. Introduction

Protein claims continue to grow in appeal across the globe. Consumers are looking to increase their intake of protein for general health and wellness purposes. Proteins are a vital nutritional element of the human diet needed for survival. Thus, proteins are necessary for growth and development of the body; for body maintenance and the repair and replacement of old or damaged tissues; to produce metabolic and digestive enzymes; and an essential constituent of certain hormones (WHO, 2007). Proteins are large molecules made of amino acids (AA); containing various amounts of 20 different AA linked via peptide bonds (Wu, 2013). AA are essential precursors for the synthesis of proteins, peptides, and low-molecular weight substances (e.g., glutathione, creatine, nitric oxide, dopamine, serotonin, RNA, and DNA) with enormous physiological importance (San Gabriel and Uneyama, 2013; Wu, 2013).

The nutritional quality of protein, also known as that participates to the global nutritive value of the product, depends on its AA content and its physiological application after digestion. AA Accessibility varies with protein source, processing methods, and interaction with other components of food like fat and minerals (Maurya and Kushwaha, 2019). Proteins are found in animal and plant foods, with where major conventional sources of the diet in developing and developed countries are cereals, meat, pulses, milk and dairy, fish, seafood, and eggs; however could not be enough in the coming decades. Therefore, it is important to search for alternative sources of high-quality protein for human consumption (Maurya and Kushwaha, 2019). Edible insects are an important and promising food resource to be developed, because they contain high-quality protein, vitamins, and AA for humans (da Silva, de Oliveira, da Rocha, and Prentice, 2020). Moreover, pulse protein isolates, used as ingredients in food formulations, increase protein nutritional value. These alternative protein sources have been used by different authors to produce enriched foods, with most baked products as breads (Roncolini et al., 2020; Osimani et al.,2018) or snacks (García-Segovia, Igual, Noguerol, Martínez-Monzó,

2020; Igual, García-Segovia and Martínez-Monzó, 2020; Azzollini, Derossi, Fogliano, Lakemond and Severini, 2018).

Alternative protein products are growing in popularity because of consumers trying to change their diets to lead a more sustainable lifestyle. Often consumers are motivated to do so because of the health benefits associated with such eating and drinking habits. Bread is a staple food throughout Europe and western countries; it is obtained from the baking of leavened dough commonly prepared with wheat flour, water, and a leavening agent, with or without the addition of salt and other ingredients (Roncolini et al., 2020). Nowadays, a wide range of breads are available, and consumers are receptive to innovative proposals as protein enriched bread.

Although the number of studies using edible insects or pea protein isolates, as a source of protein is increasing, there is still a need for further investigations on accessibility of the protein according to their AA composition and their digestibility. Therefore, we aimed to study the AA release from breads enriched with *Alphitobius diaperinus*, *Tenebrio molitor,* or pea protein during *in vitro* gastrointestinal digestion.

2. Material and methods

2.1. Raw materials

Commercial wheat bread flour, salt, water, and fresh yeast (*Saccharomyces cerevisiae*) were purchased from a local supermarket (Alcampo, Valencia, Spain). Pea protein powder (P) (Nutralys S85F) was supplied by Roquette S.L. (Spain). *Alphitobius diaperinus* (AD) and *Tenebrio molitor* (TM) powders were supplied by Entopure (Netherlands). In addition, ascorbic acid (Panreac, Spain) was used in formulations. Crude protein of pea protein, *Alphitobius diaperinus* and *Tenebrio molitor* powders were 78.72, 57.6 and 53.4%, respectively (García-Segovia et al., 2020).

Two different powder concentrations of P, AD, and TM (5 and 10%) were used to produce experimental breads. Moreover, breads produced with sole wheat bread flour were used as the control (CB). Wheat flour (60.02%), water (36.01%), fresh yeast (3.00%), salt

(0.96%), and ascorbic acid (0.01%) were the basic ingredients. Experimental doughs contained 5% or 10% less wheat flour, replaced by P, AD or TM powders, in comparison with control bread. Therefore, experimental breads after production were named as P5B, P10B, AD5B, AD10B, TM5B and TM10B.

The ingredients were mixed in a food processor (Kenwood chef classic, KM400/99 plus, Kenwood Corporation, Tokyo, Japan), kneaded for 5 min at low speed (speed 2). Dough samples were fermented for 10 minutes at 40°C in an oven (Convotherm OES 6.06 mini CC, CONVOTHERM Elektrogeräte GMBH, Eglfing, Germany). The breads were shaped by hand into 70 g weighted pieces and left to stand for 15 min at 25ºC. The pieces were baked at 170°C for 20 min in the oven described before. Breads were cooled for 1 h at 25ºC.

2.2. *In vitro* digestion

Sample *in vitro* digestibility was assessed by the standardised static *in vitro* digestion method suitable for food (COST INFOGEST network) proposed by Minekus et al. (2014) in triplicate. The *in vitro* digestion (D) protocol is summarised in Figure 1, following the four steps: *oral phase*, mixing the sample and simulated salivary fluid (SSF) (1:1, w/v) with human salivary α-amylase and $CaCl_2$ at pH 7 and 37ºC for 2 min; *gastric phase* (GP), mixing the oral bolus and simulated gastric fluid (SGF) (1:1) with pepsin from porcine gastric mucosa and $CaCl_2$ at pH 3 and 37ºC for 2 h; *intestinal phase* (IP), mixing the gastric chyme and simulated intestinal fluid (SIF) (1:1) with enzymes (pancreatin from porcine pancreas), fresh bile and $CaCl_2$ at pH 7 and 37ºC for 2 h; *filtration phase*, centrifuging at 2,600 x g for 30 min and filtering through a 1 µm glass-fibre membrane. For pH adjustment in each phase NaOH and HCl was used. SSF, SGF and SIF were prepared according Minekus et al. (2014). Samples were collected and freeze-dried using protease inhibitor when necessary, according procedures recommended by Minekus et al., (2014).

2.3. Amino acids (AA) determination

Free AA in the bread samples before (B) and after each phase of digestion (GP, IP, and D) were analysed according to Aristoy and Toldrá (1991) in triplicate. Samples were homogenised with 0.01 N HCl:Bread (1:5) and centrifuged in the cold (4 ºC) at 10,000 g for 20 min. The supernatant was filtered through a 0.45 µm membrane. A 300 µL plus 50 µL of an internal standard solution (alfa-aminobutyric, 2.5 mM), were deproteinised with 875 µL of acetonitrile. The 300 µL of supernatant was derivatised according to the method of Bidlingmeyer, Cohen, Tarvin, and Frost (1987). Derivatised samples were analysed on a Waters HPLC system with a variable UV detector at 254 nm in a Nova Pack C18 column (3.9 x 300 mm, 5 µm) (Water Corporation, MA, USA). The separation was achieved in 65 min at 52 °C using a gradient between 70 mM sodium acetate at pH 6.55 containing 2.5% of acetonitrile and water-acetonitrile-methanol, 40:45:15 (v/v/v) as described by Flores, Aristoy, Spanier, and Toldrá (1997). AA present in tap water and the reagents in each gastrointestinal phase were also analysed as blank and corrected in the AA fraction.

2.4. Statistical analysis

Analysis of variance (ANOVA), with a confidence level of 95% (p<0.05), using Statgraphics (Centurion XVII Software, version 17.2.04) was applied to evaluate the differences among breads samples and digestion phase.

3. Results and Discussion

The action of the gastrointestinal digestive phases is to reduce most dietary protein to a mixture of free AA, dipeptides, and tripeptides, available for absorption in the body (Erickson and Kim, 1990). There are over 300 AA in nature, but only 20 (α-AA) serve as the building blocks of protein. However, non-protein α-AA (e.g., citrulline) and non-α AA (e.g., taurine and β-alanine) also play important roles in cell metabolism (Curis, Crenn, and Cynober, 2007; Hu, et al. 2008). Figure 2 shows total free AA mean values and

standard deviation of B, GP, IP, and D. The addition of edible insects (AD or TM) significantly increased ($p < 0.05$) the total free AA in breads, with most seen in TM10B (338.2 mg/100g); after GP digestion, there was little AA release. The digestion of dietary protein begins in the stomach where proteolysis produces large polypeptides, few smaller peptides, and minimal free AA (Joye, 2019). Thus, breads after IP showed significantly greater ($p < 0.05$) total free AA content than breads after GP. Since pancreatin react with the digest to break down proteins and produce amino acids in intestinal processing while pepsin in gastric processing catalyse breakdown of proteins in fragment of amino acids (Boland 2017). The highest free AA increase was seen in IP. Whereas, after filtration following *in vitro* digestion D, there was another total free AA significant increase ($p < 0.05$), probably for centrifuging that force the AA separation before filtration; this was seen for all samples except to TM5B.There were significant differences ($p < 0.05$) in samples before and after *in vitro* digestion. After digestion, samples from highest to lowest free AA content were P10B > TM10B > AD10B > P5B ≈ AD5B ≈ TM5B > CB. Therefore, using P, AD, or TM at any assay level presented significantly freer AA, accessible for body absorption. The order of samples shows an effect of concentration (10 > 5%) in bread, giving more free AA after *in vitro* gastrointestinal digestion. Real free AA amounts for body absorption could be more, since *in vitro* static digestion systems do not take the brush border peptidases into account (Huang, Pan, Zhong, Yan, Duan, and Jia, 2018; Cordelino, Inamdar, Vickers, Marti, and Ismail, 2019). These brush border peptidases N-hydrolyse short oligopeptides by sequentially removing N-terminal amino acids (Erickson and Kim, 1990), hence the free AA could be higher. The effect of concentration (10 > 5%) in bread could be related with the act that bread with an addition of 10% has a higher final amount of protein. It was observed by Oliveira, Lucas, Cadaval and Salas-Mellado (2017) in bread with a 10% addition of flour from cinereous cockroach (*Nauphoeta cinerea*),

It is widely accepted that nine out of the twenty naturally occurring AA are indispensable (IAA). These AA, histidine, isoleucine, leucine, lysine, methionine, phenylalanine,

159 threonine, tryptophan, and valine cannot be synthesised by an animal organism from the
160 materials, ordinarily available to the cells at a speed commensurate with the demands
161 for normal growth, therefore need to be part of a healthy balanced diet (Reeds, 2000).
162 Figure 3 shows total free IAA mean values and standard deviation of B, GP, IP, and D.
163 TM10B presented the significantly greatest ($p < 0.05$) free IAA content in bread, the same
164 trend was observed for total free AA (Figure 2). CB and P5B showed significantly lower
165 values of free IAA than the other breads. There are significant differences ($p < 0.05$)
166 among the phases (B, GP, IP, and D) for all samples except to CB. The greater difference
167 is observed between GP and IP in all cases except to AD5B, which presented a major
168 difference between IP and D. According to values of total IAA of breads after *in vitro*
169 gastrointestinal digestion, there are three groups significantly different ($p < 0.05$). The
170 first is formed by P10B with the highest values of free total IAA accessible for body
171 absorption. The second group has TM10B and P5B, and the last group has AD10B,
172 TM5B, CB, and AD5B. Probably, protein content of P, AD and TM is the main responsible
173 of this distribution of breads. Since P showed the higher protein content according
174 (García-Segovia et al., 2020).
175 Table 1 shows the content of free IAA in each phase. According to the studied phases,
176 breads of D showed higher values of individual free IAA, likewise with total AA and total
177 IAA. Free histidine was not detected in GP, IP, and D for CB. However, all experimental
178 breads showed free histidine in all phases. TM10B presented the significantly highest (p
179 < 0.05) free histidine value after *in vitro* gastrointestinal digestion. Free tryptophan was
180 not detected in GP and IP for all samples. However, all samples presented free
181 tryptophan in D. This fact may be due to necessity for a force to release tryptophan.
182 Either force of the centrifuge and filtration (*in vitro*) or force of absorption in the intestine
183 (*in vivo*). Free tryptophan was significantly higher ($p< 0.05$) in P10B than the other
184 samples. Free threonine and valine in experimental breads, after *in vitro* gastrointestinal
185 digestion was significantly higher ($p < 0.05$) than the control. However, there is no effect
186 of concentration of P, TM, or AD on free threonine content, whereas free valine content

was higher in breads enriched with 10% of P, TM, or AD than samples with 5%. Free valine, methionine, and isoleucine content of samples in GP were not detected, but subsequently, these free IAA in IP increased significantly ($p < 0.05$). The highest content of free methionine, lysine, and isoleucine after *in vitro* gastrointestinal digestion was achieved by P10B. Leucine and phenylalanine were the highest values at the end of *in vitro* gastrointestinal digestion than the other IAA. Besides, P10B achieved significantly higher ($p < 0.05$) free leucine and phenylalanine values than the other breads. Since P presented the higher protein content (García-Segovia et al., 2020), P10B is the higher concentration and P showed higher content of leucine and phenylalanine than AD and TM according other studies (Janssen, Vincken, van den Broek, Fogliano and Lakemond, 2017; Leterme, Monmart and Baudart, 1990)

Tables 2 shows mean values of dispensable AA in studied breads for each phase of *in vitro* gastrointestinal digestion. According to Reeds (2000) a dispensable AA is defined as one that can be synthesised *de novo* from a non-AA source of nitrogen and an appropriate carbon source. Table 2 includes aspartic acid, glutamic acid, serine, glycine, alanine, proline, tyrosine cysteine, hydroxyproline, asparagine, glutamine, β-alanine, taurine, GABA, citrulline, anserine, and arginine. For each sample, free aspartic acid content was significantly higher ($p < 0.05$) in B than in GP. AD10B presented the highest values of free aspartic acid accessible for body absorption. Experimental breads, after *in vitro* gastrointestinal digestion showed significantly higher ($p < 0.05$) free glutamic acid values than CB, especially edible insect samples, TM10B and AD10B. Free serine values of the different phases did not follow a clear trend, in CB, P5B, TM5B, and AD10B there are significant differences ($p < 0.05$) among GP, IP, and D, whereas P10B, TM10B, and AD5B not showed differences. This behaviour could be provoked by the existence of serine protease inhibitors in both insects and pea (Schoofs and Salzet, 2002; Shingles, Woodrow and Grodzinski, 1984). Among studied phases, significantly higher ($p < 0.05$) content of free glycine in IP was observed for all samples, yet after filtration, free glycine decreased. After *in vitro* gastrointestinal digestion, CB showed the significantly lowest (*p*

215 < 0.05) value of free alanine than other samples. Experimental breads ranked from
216 highest to lowest free alanine content after digestion are AD10B > TM10B > P10B >
217 AD5B > P5B > TM5B. The order of samples shows a trend of AD, TM, or P concentration
218 (10 > 5%) in bread. Comparing differrent studies about amino acids in AD, TM and P
219 (Janssen et al., 2017; Leterme et al.,1990) the alanine content in AD and TM was higher
220 than P. This could be the reason for the higher alanine content in digested bread with
221 AD and TM. Moreover, alanine content in AD, TM and P was higher than alanine content
222 in wheat flour (Shoup, Pomeranz and Deyoe, 1966). Therefore, experimental breads
223 showed higher values of free alanine content in digested breads than CB. The behaviour
224 of proline is remarkable over the other studied AA for its free content in *in vitro*
225 gastrointestinal digestion. While studied free AA showed a notable increase in IP, free
226 proline not show a clear trend, showing breads significantly ($p < 0.05$) higher free proline
227 than samples in IP or D. Yoshioka, Erickson, and Kim (1988) indicated the intestinal
228 brush border membrane contains at least four peptidases that have high hydrolytic rates,
229 and peptides containing a proline residue at the site of cleavage. Dipeptidyl
230 aminopeptidase IV and aminopeptidase P cleave prolyl peptides from the amino
231 terminus, whereas angiotensin-converting enzyme and carboxypeptidase P work
232 synergistically to hydrolyse prolyl peptides from the carboxy terminal end. The
233 specificities of these enzymes are believed to be complementary to those of pancreatic
234 proteases, which have little or no ability to hydrolyse peptide bonds involving proline. As
235 previously stated, *in vitro* static digestion systems do not take the brush border
236 peptidases into account (Huang, Pan, Zhong, Yan, Duan, and Jia, 2018; Cordelino,
237 Inamdar, Vickers, Marti, and Ismail, 2019), thus here, changes of free proline in IP and
238 D, that would be observed in vivo, were not detected in such in vitro models. Moreover,
239 using edible insects in formulations significantly increases ($p < 0.05$) the free proline
240 content in breads, especially when using TM. In this study there is an effect of edible
241 insect concentration (10 > 5%) on free proline content; with free tyrosine being the
242 highest in IP, observed in Table 2, and notably higher values than GP. P10B and TM10B

showed significantly higher (p < 0.05) free tyrosine content accessible for body absorption than the other samples. Free cysteine content after *in vitro* gastrointestinal digestion was higher in CB than experimental breads. Probably due to the higher cysteine content in wheat flour compared to AD, TM and P powders, according to the cysteine contents shown by other authors (Janssen et al., 2017; Leterme et al., 1990; Shoup et al., 1966). Hydroxyproline showed a similar trend to proline before *in vitro* digestion. Using edible insects in formulations significantly increases (p < 0.05) the free hydroxyproline content in breads, presenting the highest content in AD10. Although, experimental breads showed significantly higher (p <0.05) values of free asparagine, CB presented the significantly highest (p < 0.05) free asparagine content after *in vitro* gastrointestinal digestion. Table 2 shows the free asparagine differences in IP and D of CB and the experimental breads was considerable. Free glutamine content in B was significantly higher (p < 0.05) in P10B, TM5B, TM10B, AD5B, and AD10B. However, samples after *in vitro* digestion did not show significant differences (p > 0.05) in free glutamine values among samples. Likewise, alanine after *in vitro* gastrointestinal digestion, showed CB had the significantly lowest (p < 0.05) value of free β-alanine than the other samples. Using edible insects in formulations significantly increases (p < 0.05) the free taurine content in breads, presenting the highest content in AD10, as with hydroxyproline. However, there is no significant difference (p > 0.05) between AD10 and CB in samples after *in vitro* gastrointestinal digestion.

The highest values of free citrulline were observed in CB in all phases. It was reported that citrulline, which occur in yeast, acted as major precursors during thermal generation leading to 2-actyl-1-pyrroline (Schieberle, 1990), which is the primary odorant of bread crusts (Cho and Peterson, 2010). AD10B showed the significantly highest (p < 0.05) values of free GABA and anserine after *in vitro* gastrointestinal digestion, whereas CB presented the significantly lowest (p < 0.05) free content of these AA in D. After *in vitro* gastrointestinal digestion, free arginine accessible for body absorption was significantly higher (p < 0.05) in experimental breads than CB. According to Barbul, Sisto,

Wasserkrug, and Efron (1981) arginine is a safe nutritional stimulator of lymphocyte immune reactivity in healthy human beings. Therefore, P10B, with highest free arginine content in D, can be used as a functional product for those to boost their immune activity.

4. Conclusions

In this study the AA release from breads enriched with *Alphitobius diaperinus*, *Tenebrio molitor*, or pea protein during *in vitro* gastrointestinal digestion was evaluated. The highest AA release from bread during digestion mostly occurred in intestinal phase. Using pea protein, *Alphitobius diaperinus,* and *Tenebrio molitor* powders at any assay level presented a significantly higher value of total free AA accessible for body absorption. There is an effect of ingredient enrichment concentration (10 > 5%) in bread on total AA release after *in vitro* gastrointestinal digestion. Bread enrichment with 10% pea protein showed the highest values of free AA values after *in vitro* gastrointestinal digestion. However, depending on the target AA, AD10B and TM10B also presented the highest values for glutamic acid, TM10B for histidine and proline, and AD10B for aspartic acid.

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Figure captions

Figure 1. Flow diagram of simulated *in vitro* digestion method. SSF, SGF, and SIF are Simulated Salivary Fluid, Simulated Gastric Fluid, and Simulated Intestinal Fluid, respectively.

Figure 2. Total free amino acids mean values (mg / 100 gbread) and standard deviation of studied breads and these after gastric, intestinal, and completed gastrointestinal digestion. Letters indicate homogeneous groups established by the ANOVA ($p < 0.05$) for kind of bread (A-F) and for sample state (a-d). CB: Control Bread; P5B: 5% Pea protein Bread; P10B: 10% Pea protein Bread; TM5B: 5% *Tenebrio molitor* Bread; TM10B: 10% *Tenebrio molitor* Bread; AD5B: 5% *Alphitobius diaperinus* Bread; AD10B: 10% *Alphitobius diaperinus* Bread. B, GP, IP, and D are Bread, Gastric Phase, Intestinal Phase, and Digested sample, respectively.

Figure 3. Total free indispensable amino acids mean values (mg / 100 g_{bread}) and standard deviation of studied breads and these after gastric, intestinal and completed gastrointestinal digestion. Letters indicate homogeneous groups established by the ANOVA ($p< 0.05$) for kind of bread (A-F) and for sample state (a-d). CB: Control Bread; P5B: 5 % Pea protein Bread; P10B: 10 % Pea protein Bread; TM5B: 5 % *Tenebrio molitor* Bread; TM10B: 10 % *Tenebrio molitor* Bread; AD5B: 5 % *Alphitobius diaperinus* Bread; AD10B: 10 % *Alphitobius diaperinus* Bread. B, GP, IP and D are Bread, Gastric Phase, Intestinal Phase and Digested sample, respectively.

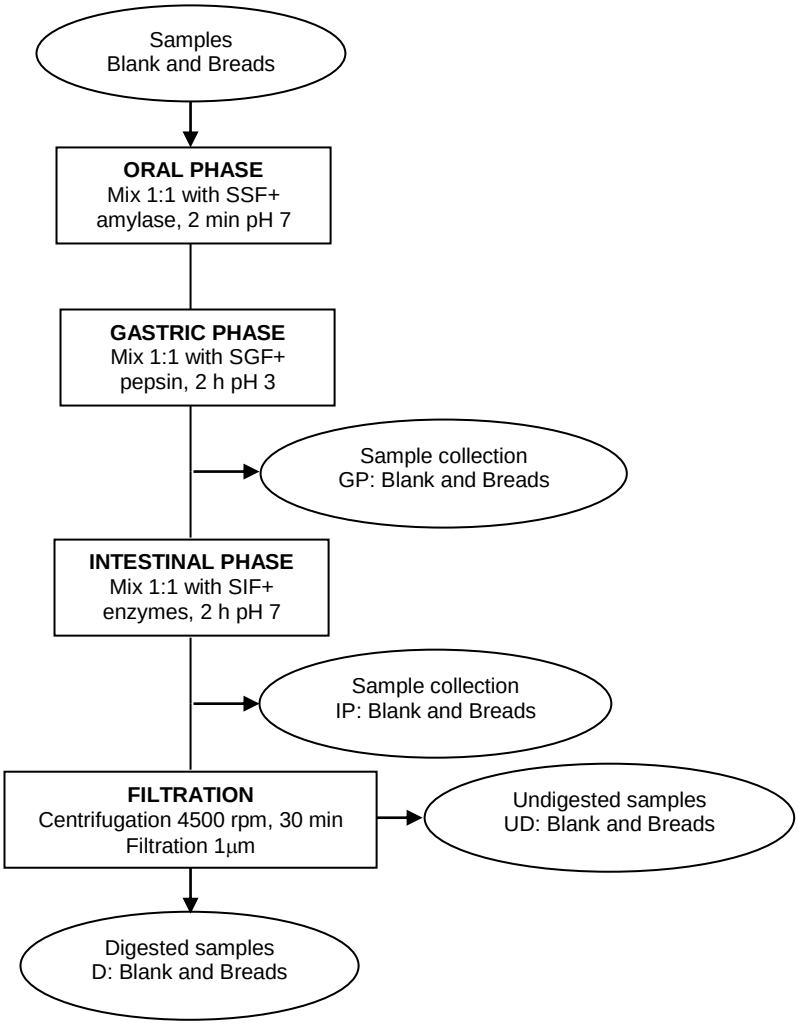

Figure 1.

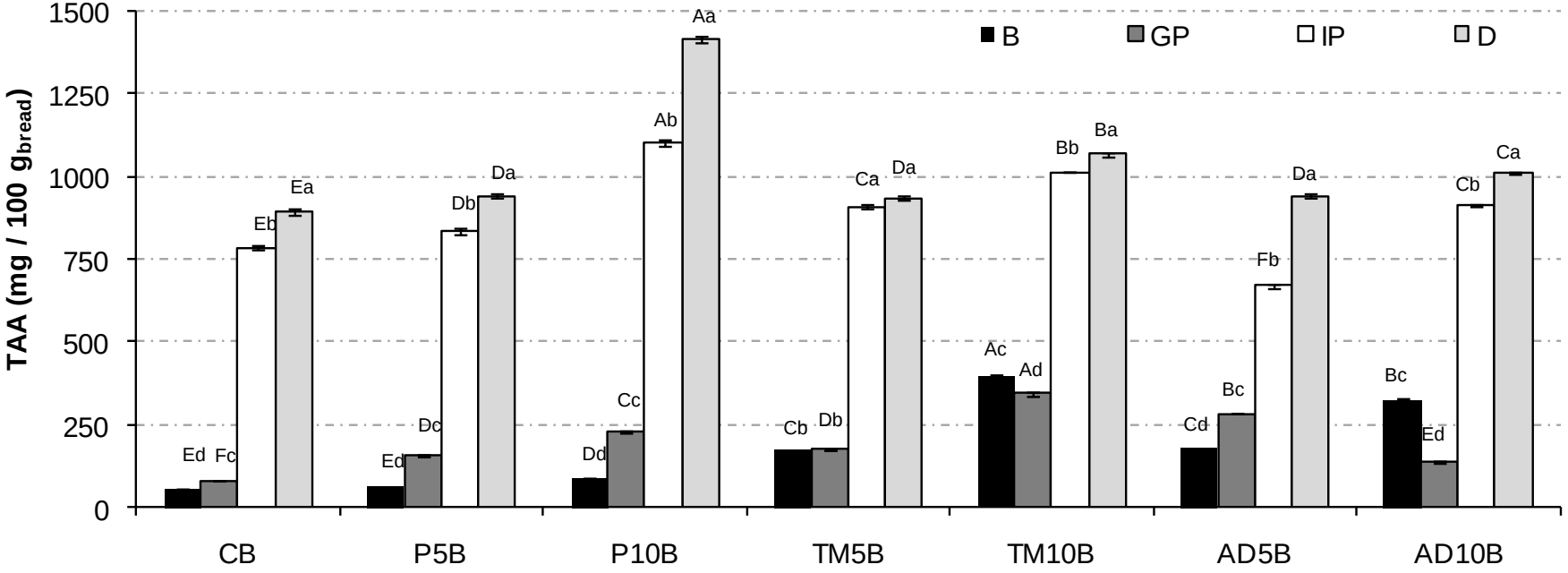

Figure 2.

Figure 3

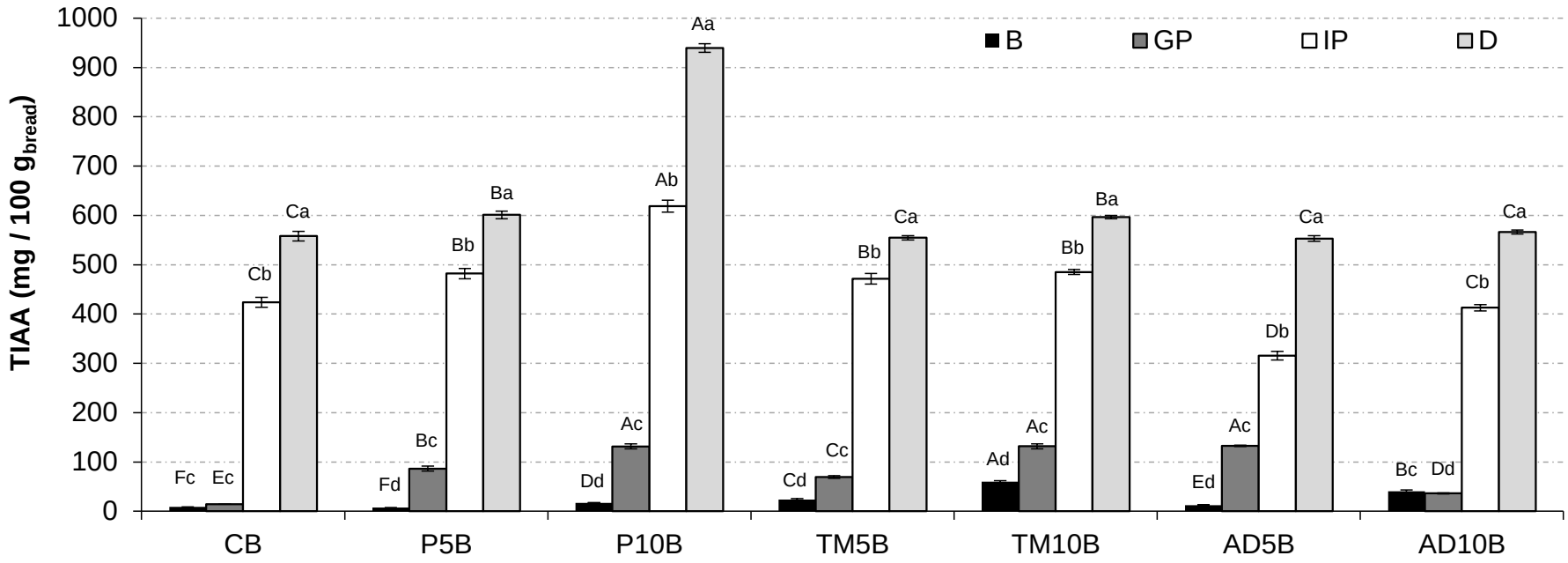

Figure 3.

Table 1. Indispensable amino acids mean values (mg / 100 g_{bread}) and standard deviation in brackets of studied samples.

Samples	Phase	Histidine	Tryptophan	Threonine	Valine	Methionine	Lysine	Isoleucine	Leucine	Phenylalanine
CB	B	0.51 (0.09)^{Da}	1,50 (0,05)^{Cb}	0.80 (0.05)^{BCa}	1.66 (0.07)^{Cc}	0.40 (0.05)^{CDc}	1.98 (0.07)^{DEb}	0.63 (0.05)^{Cc}	1.16 (0.03)^{Cc}	0.69 (0.03)^{Bc}
	GP	0^{Eb}	0^{-b}	0.65 (0.03)^{Bb}	0^{-d}	0^{Cc}	1.34 (0.02)^{BCb}	0^{-c}	11 (2)^{Dc}	0^{Fc}
	IP	0^{Db}	0^{-b}	0.50 (0.05)^{Ebc}	5.8 (0.2)^{Eb}	3.7 (0.2)^{CDb}	10.8 (0.6)^{CDa}	9.1 (1.2)^{Cb}	181 (8)^{BCb}	207 (9)^{CDb}
	D	0^{Eb}	45 (2)^{BCa}	0.47 (0.05)^{Dc}	7.9 (0.3)^{Da}	9.3 (0.8)^{Ca}	11.5 (0.5)^{Ca}	14 (2)^{Ca}	214 (5)^{Ba}	251 (10)^{CDa}
P5B	B	0.62 (0.08)^{Dc}	0,27 (0,02)^{Cb}	0.46 (0.03)^{Dc}	1.74 (0.08)^{BCb}	0.36 (0.02)^{Dc}	2.11 (0.09)^{DEb}	0.54 (0.05)^{Cc}	0.96 (0.09)^{Cd}	0.50 (0.05)^{Bd}
	GP	0.24 (0.08)^{Dd}	0^{-b}	0.64 (0.04)^{Bbc}	0^{-b}	0.021 (0.009)^{Bc}	1.00 (0.05)^{Cb}	0^{-c}	38 (3)^{Cc}	45 (5)^{Cc}
	IP	1.21 (0.05)^{Ca}	0^{-b}	0.76 (0.03)^{Db}	15 (2)^{Ca}	6.6 (0.3)^{Ab}	14 (2)^{BCa}	15.3 (1.2)^{Bb}	190 (5)^{Bb}	236 (9)^{Bb}
	D	0.98 (0.07)^{Db}	28,9 (1,2)^{Da}	1.6 (0.2)^{Aa}	17 (2)^{BCa}	11.9 (0.8)^{ABa}	14 (2)^{Ca}	18.7 (0.9)^{BCa}	226 (5)^{Ba}	278 (8)^{Ba}
P10B	B	6.5 (0.8)^{Ca}	1,40 (0,04)^{Cb}	0.94 (0.05)^{Bc}	2.2 (0.2)^{BCb}	0.52 (0.04)^{Cc}	2.9 (0.2)^{CDb}	0.67 (0.08)^{Cc}	1.27 (0.08)^{Cd}	0.77 (0.08)^{Bd}
	GP	0.26 (0.04)^{Dc}	0^{-b}	0.36 (0.04)^{Cd}	0^{-b}	0.15 (0.02)^{Ac}	1.98 (0.09)^{ABb}	0-^{c}	51 (7)^{Bc}	73 (5)^{Ac}
	IP	0.91 (0.08)^{Cbc}	0^{-b}	1.16 (0.04)^{Cb}	17 (2)^{ABCa}	6.8 (0.7)^{Ab}	26 (3)^{Aa}	19 (2)^{Ab}	224 (7)^{Ab}	318 (11)^{Ab}
	D	1.43 (0.09)^{Cb}	54 (4)^{Aa}	1.53 (0.08)^{ABa}	22 (3)^{Aa}	13.1 (0.8)^{Aa}	27 (4)^{Aa}	30 (4)^{Aa}	324 (10)^{Aa}	458 (10)^{Aa}
TM5B	B	8.2 (0.9)^{Ca}	5,3 (0,5)^{Bb}	0.85 (0.05)^{BCb}	0.49 (0.05)^{Db}	0.77 (0.04)^{Bc}	3.7 (0.8)^{Cb}	1.3 (0.2)^{BCb}	2.3 (0.2)^{Bd}	1.02 (0.09)^{Bd}
	GP	2.74 (0.08)^{Cb}	0^{-b}	0.62 (0.03)^{Bc}	0^{-b}	0^{Cc}	0.94 (0.09)^{Cbc}	0^{-b}	34 (4)^{Cc}	29 (3)^{Dc}
	IP	1.48 (0.07)^{Cb}	0^{-b}	1.36 (0.07)^{Ba}	15.5 (0.9)^{BCa}	6.7 (0.8)^{Ab}	0.58 (0.05)^{Ec}	16 (2)^{Ba}	199 (9)^{Bb}	224 (8)^{BCb}
	D	1.66 (0.09)^{Cb}	41 (3)^{Ca}	1.51 (0.09)^{ABa}	14.8 (0.7)^{Ca}	11.3 (0.9)^{Ba}	9 (2)^{Ca}	17 (3)^{BCa}	215 (3)^{Ba}	240 (6)^{CDa}
TM10B	B	21 (2)^{Aa}	9,8 (1,2)^{Ab}	0.16 (0.02)^{Ec}	9.5 (0.5)^{Ab}	1.23 (0.07)^{Ac}	8.1 (0.7)^{Ac}	3.0 (0.8)^{Ac}	3.17 (0.08)^{Ad}	3.4 (0.8)^{Ad}
	GP	5.03 (0.08)^{Ab}	0^{-c}	0.22 (0.05)^{Dc}	0^{-c}	0^{Cd}	0.16 (0.04)^{Dd}	0^{-c}	61 (5)A^{Bc}	62 (5)^{Bc}
	IP	3.4 (0.8)^{Ab}	0^{-c}	1.64 (0.09)^{Aa}	21 (2)^{Aa}	5.8 (0.7)^{ABb}	16 (2)^{Ba}	17.4 (1.2)^{ABb}	195 (9)^{Bb}	218 (4)^{BCb}
	D	3.4 (0.2)^{Ab}	48 (4)^{Ca}	1.3 (0.2)^{Bb}	21 (2)^{Aa}	10.8 (0.4)^{BCa}	11.7 (1.2)^{Cb}	21 (2)^{Ba}	216 (7)^{Ba}	257 (5)^{Ca}
AD5B	B	0.11 (0.02)^{Db}	1,42 (0,09)^{Cb}	0.73 (0.08)^{Cc}	2.35 (0.09)^{Bd}	0.54 (0.06)^{Cc}	5.1 (0.9)^{Bb}	0.75 (0.04)^{BCc}	1.19 (0.03)^{Cd}	0.62 (0.07)^{Bd}
	GP	0^{Eb}	0^{-b}	0.90 (0.03)^{Ac}	0^{-c}	0^{Cc}	2.4 (0.8)^{Ac}	0^{-c}	64 (5)^{Ac}	62 (2)^{Bc}
	IP	2.20 (0.08)^{Ba}	0^{-b}	1.41 (0.05)^{Bb}	10.2 (0.9)^{Db}	3.2 (0.7)^{Db}	4.2 (0.5)^{Ebc}	7.5 (0.8)^{Cb}	150 (8)^{Db}	131 (8)^{Eb}
	D	2.18 (0.05)^{Ba}	40 (2)^{Ca}	1.62 (0.07)^{Aa}	14.3 (0.8)^{Ca}	9.5 (0.6)^{Ca}	8.6 (1.2)^{Ca}	14.8 (1.2)^{Ca}	220 (4)^{Ba}	239 (7)^{Da}
AD10B	B	16 (2)^{Ba}	5,3 (0,9)^{Bb}	1.56 (0.07)^{Aa}	9.0 (0.5)^{Ab}	0.76 (0.07)^{Bc}	1.38 (0.08)^{Ec}	1.48 (0.06)^{Bc}	1.7 (0.3)^{BCd}	3.35 (0.09)^{Ac}
	GP	3.15 (0.08)^{Bb}	0^{-c}	0^{Ec}	0^{-c}	0^{Cd}	0.74 (0.04)^{CDc}	0^{-c}	20 (2)^{Dc}	11.1 (1.2)^{Ec}
	IP	2.52 (0.09)^{Bb}	0^{-c}	1.44 (0.08)^{Ba}	19 (2)^{ABa}	4.8 (0.2)^{BCb}	9.6 (0.5)^{Db}	14 (2)^{Bb}	166 (5)^{CDb}	192 (3)^{Db}
	D	2.18 (0.05)^{Bb}	49 (3)^{ABa}	0.88 (0.07)^{Cb}	20 (2)^{ABa}	11.4 (0.5)^{Ba}	19 (3)^{Ba}	19 (3)^{BCa}	193 (5)^{Ca}	247 (5)^{CDa}

Letters indicate homogeneous groups established by the ANOVA (p< 0.05) within column for kind of bread (A-E) and for sample state (a-d). CB: Control Bread; P5B: 5 % Pea protein Bread; P10B: 10 % Pea protein Bread; TM5B: 5 % *Tenebrio molitor* Bread; TM10B: 10 % *Tenebrio molitor* Bread; AD5B: 5 % *Alphitobius diaperinus* Bread; AD10B: 10 % *Alphitobius diaperinus* Bread. B, GP, IP and D are Bread, Gastric Phase, Intestinal Phase and Digested sample, respectively.

Table 2. Dispensable amino acids mean values (mg / 100 g_{bread}) and standard deviation in brackets of studied samples.

Samples	Phase	Aspartic acid	Glutamic acid	Serine	Glycine	Alanine	Proline	Tyrosine	Cysteine
CB	B	3.0 (0.3)^{Da}	12.6 (1.2)^{Da}	1.07 (0.04)^{Eb}	2.53 (0.09)^{Cc}	4.48 (0.09)^{Cb}	1.9 (0.2)^{Da}	0.56 (0.05)^{Db}	9.9 (0.8)^{ABCDc}
	GP	0^{Gb}	6.3 (0.9)^{Cb}	0.72 (0.03)^{Ec}	1.04 (0.08)^{Ec}	1.53 (0.04)^{Fc}	0.54 (0.07)^{Dd}	9.66 (0.09)^{Db}	31 (3)^{Ab}
	IP	0^{Eb}	6.0 (0.8)^{Fbc}	2.11 (0.09)^{Aa}	44 (3)^{Aa}	7.5 (0.9)^{Ca}	0.93 (0.08)^{Db}	115 (6)^{Ca}	106 (8)^{Aa}
	D	0^{Eb}	3.6 (0.8)^{Dc}	1.17 (0.07)^{Cb}	25 (2)^{ABb}	5.1 (0.6)^{Cb}	0.64 (0.06)^{Dcd}	122 (8)^{Da}	108 (6)^{Aa}
P5B	B	3.2 (0.4)^{Da}	19 (2)^{CDb}	1.24 (0.05)^{Ec}	2.72 (0.09)^{Cc}	5.0 (0.3)^{Cb}	1.8 (0.2)^{Da}	0.58 (0.04)^{Dc}	7.2 (0.8)^{Db}
	GP	0.70 (0.06)^{Fc}	11.1 (0.9)^{Bc}	1.09 (0.06)^{Dc}	1.21 (0.04)^{DEc}	3.0 (0.2)^{DEc}	1.15 (0.09)^{Db}	24 (2)^{Cb}	19 (3)^{BCb}
	IP	1.87 (0.07)^{Db}	36 (2)^{DEa}	2.60 (0.09)^{Aa}	36 (4)^{ABa}	9.1 (0.9)^{BCa}	1.47 (0.07)^{Db}	132 (10)^{Ca}	59 (7)^{Ba}
	D	1.04 (0.02)^{Dc}	31 (3)^{Ca}	1.78 (0.08)^{Bb}	23 (3)^{Bb}	8.0 (0.7)^{Ba}	1.28 (0.06)^{Db}	138 (6)^{CDa}	66 (5)^{Ca}
P10B	B	5.8 (0.7)^{Ca}	28 (2)^{Bb}	1.71 (0.2)^{Db}	3.1 (0.2)^{Cc}	7.0 (0.3)^{Cb}	2.88 (0.09)^{Da}	1.36 (0.07)^{Dd}	9.1 (1.2)^{CDc}
	GP	1.17 (0.08)^{Dc}	25 (4)^{Ab}	2.39 (0.09)^{Ca}	1.38 (0.07)^{Dc}	2.89 (0.09)^{Ec}	1.84 (0.08)^{Dc}	32 (3)^{Cc}	19 (4)^{Bc}
	IP	2.6 (0.2)^{ABb}	61 (3)^{Aa}	2.57 (0.08)^{Aa}	43 (5)^{ABa}	10.1 (1.2)^{BCa}	2.13 (0.09)^{Db}	165 (9)^{Bb}	64 (8)^{Bb}
	D	1.44 (0.08)^{Bc}	30 (2)^{Cb}	2.51 (0.09)^{Aa}	26 (2)^{ABb}	9.2 (0.9)^{ABa}	2.32 (0.06)^{Db}	208 (10)^{Aa}	79 (5)^{Ba}
TM5B	B	5.2 (0.4)^{Ca}	26 (3)^{BCb}	1.99 (0.07)^{Cb}	3.6 (0.2)^{Cc}	7.7 (1.2)^{Ca}	48 (4)^{Ca}	9.4 (0.5)^{Cc}	12 (2)^{ABb}
	GP	1.36 (0.07)^{Cc}	2.1 (0.2)^{Cc}	2.18 (0.2)^{Cb}	1.63 (0.09)^{Cc}	4.3 (0.9)^{CDb}	38 (3)^{BCa}	32 (4)^{Cb}	12.5 (0.9)^{CDb}
	IP	2.18 (0.09)^{CDb}	43 (5)^{CDa}	2.78 (0.09)^{Aa}	34 (4)^{Ba}	10.2 (1.2)^{Ba}	40 (8)^{Ca}	170 (9)^{Ba}	57 (6)^{Ba}
	D	1.35 (0.04)^{Bc}	36 (2)^{BCa}	1.92 (0.09)^{Bb}	16 (2)^{Cb}	7.5 (0.8)^{Ba}	35 (5)^{Ca}	165 (3)^{Ba}	52 (4)^{Da}
TM10B	B	13 (2)^{Aa}	53 (3)^{Aa}	2.86 (0.08)^{Aa}	6.7 (0.9)^{Ac}	19 (3)^{Ba}	116 (8)^{Aa}	33 (6)^{Ac}	12.3 (1.2)^{Ac}
	GP	1.59 (0.08)^{Bb}	3.2 (0.9)^{Cb}	3.39 (0.2)^{Aa}	2.31 (0.08)^{Bc}	7.4 (0.9)^{Ac}	83 (6)^{Ab}	68 (7)^{Ab}	30 (3)^{Ab}
	IP	2.4 (0.2)^{Bcb}	51 (4)^{BCa}	3.47 (0.2)^{Aa}	37 (3)^{ABa}	14.2 (1.2)^{Aab}	76 (7)^{Ab}	207 (10)^{Aa}	51 (6)^{BCa}
	D	1.47 (0.09)^{Bb}	45 (3)^{Aa}	2.24 (0.09)^{Aa}	16 (3)^{Cb}	10.3 (0.8)^{Abc}	68 (4)^{Ab}	204 (9)^{Aa}	51 (4)^{Da}
AD5B	B	8.4 (0.9)^{Ba}	33 (4)^{Ba}	1.76 (0.08)^{CDb}	5.06 (0.07)^{Bc}	8.1 (1.2)^{Cab}	50 (3)^{Ca}	6.0 (1.2)^{CDd}	10.6 (0.9)^{ABCd}
	GP	1.78 (0.08)^{Ab}	6.2 (0.9)^{Cb}	2.8 (0.2)^{Ba}	2.54 (0.09)^{Ac}	6.0 (0.6)^{Bb}	45 (6)^{Bab}	42 (4)^{Bc}	22 (3)^{Bc}
	IP	2.3 (0.2)^{BCb}	30 (3)^{Ea}	2.4 (0.2)^{Aa}	40 (4)^{ABa}	10.9 (1.2)^{Ba}	34 (2)^{Cc}	127 (7)^{Cb}	38 (3)^{Cb}
	D	1.18 (0.09)^{Cb}	37 (2)^{Ba}	2.4 (0.3)^{Aa}	28 (2)^{Ab}	9.2 (0.9)^{ABa}	37 (3)^{Cbc}	151 (9)^{Ca}	63 (4)^{CDa}
AD10B	B	12.3 (0.9)^{Aa}	48 (7)^{Aa}	2.25 (0.09)^{Bb}	6.2 (0.9)^{ABb}	23 (3)^{Aa}	80 (7)^{Ba}	15 (2)^{Bb}	9.4 (0.8)^{BCDb}
	GP	0.89 (0.08)^{Ec}	13 (2)^{Bb}	0.46 (0.04)^{Ed}	1.14 (0.08)^{Ec}	5.4 (0.4)^{ACd}	34 (4)^{Cc}	25 (6)^{Cb}	6.8 (0.5)^{Db}
	IP	2.80 (0.09)^{Ab}	56 (5)^{ABa}	3.10 (0.09)^{Aa}	35 (3)^{Ba}	15.7 (1.2)^{Ab}	60 (6)^{Bb}	170 (10)^{Ba}	55 (4)^{Ba}
	D	1.69 (0.04)^{Abc}	50 (5)^{Aa}	1.43 (0.08)^{Cc}	7.6 (0.8)^{Db}	10.5 (0.9)^{Ac}	53 (4)^{Bb}	177 (6)^{Ba}	62 (5)^{CDa}

Table 2. (continued)

Samples	Phase	Hydroxiproline	Asparagine	Glutamine	β-alanine	Taurine	GABA	Citrulline	Anserine	Arginine
CB	B	0,41 (0,05)^{Ec}	0,94 (0,05)^{Ed}	1,40 (0,09)^{Ec}	0,32 (0,06)^{Ea}	0,77 (0,03)^{Eb}	0,99 (0,07)^{Dc}	3,7 (0,3)^{Ac}	0,46 (0,03)^{Bb}	5.4 (0.7)^{DEb}
	GP	0,96 (0,08)^{Bb}	3,49 (0,12)^{Ac}	1,02 (0,07)^{Fc}	0^{Eb}	0^{Db}	0^{Ed}	3,8 (0,3)^{Ac}	3,7 (0,2)^{Aa}	5.1 (0.8)^{Db}
	IP	3,1 (0,2)^{Ba}	8,3 (0,3)^{Aa}	7,6 (0,5)^{CDb}	0^{Db}	9,5 (1,2)^{ABa}	2,9 (0,2)^{Ea}	27 (4)^{Aa}	0^{Dc}	32 (2)^{Da}
	D	2.89 (0,09)^{Ba}	7,1 (0,3)^{Ab}	9,1 (0,8)^{Ba}	0^{Cb}	8,46 (0,9)^{Aa}	1,72 (0,09)^{Eb}	14 (3)^{Ab}	0^{Dc}	30 (2)^{Ea}
P5B	B	0,69 (0,07)^{DEc}	1,32 (0,08)^{Dc}	1,52 (0,07)^{Ec}	0,27 (0,03)^{Eb}	0,59 (0,05)^{Eb}	0,88 (0,06)^{Dc}	1,74 (0,09)^{Cb}	0,46 (0,03)^{Bc}	8.6 (0.7)^{Db}
	GP	1,01 (0,09)^{Bb}	0,93 (0,06)^{Dd}	1,35 (0,07)^{Ec}	0^{Ec}	0,37 (0,03)^{CDb}	0,54 (0,03)^{Dd}	0^{Ec}	2,49 (0,09)^{Bb}	7.5 (0.5)^{Cb}
	IP	2,29 (0,12)^{Ca}	3,41 (0,12)^{CDa}	7,1 (0,7)^{Db}	0,69 (0,07)^{Ca}	5,6 (0,8)^{Da}	5,2 (0,8)^{Da}	0,16 (0,02)^{Cc}	4,0 (0,3)^{Ba}	51 (3)^{Ca}
	D	2,26 (0,09)^{Ca}	2,03 (0,09)^{Db}	8,8 (0,6)^{Ba}	0,60 (0,06)^{Ba}	4,8 (0,6)^{CDa}	3,9 (0,4)^{Db}	2,3 (0,2)^{Da}	3,6 (0,2)^{Ca}	47 (2)^{BCa}
P10B	B	0,79 (0,04)^{DEd}	1,77 (0,08)^{Cc}	2,61 (0,09)^{Dc}	0,126 (0,009)^{Ec}	0,55 (0,04)^{Eb}	1,95 (0,12)^{Dc}	2,61 (0,09)^{Bb}	0,90 (0,05)^{Ad}	0.25 (0.03)^{Ed}
	GP	2,12 (0,08)^{Ac}	1,26 (0,04)^{Cd}	1,54 (0,07)^{DEd}	0^{Ed}	0,47 (0,04)^{CDb}	1,08 (0,09)^{Cd}	0,85 (0,05)^{Dc}	2,05 (0,08)^{Cc}	8.9 (0.4)^{ABc}
	IP	3,8 (0,2)^{Ab}	3,9 (0,2)^{Ba}	8,6 (0,4)^{BCb}	0,70 (0,03)^{Ca}	7,0 (0,9)^{BCDa}	8,8 (0,2)^{BCa}	7,7 (0,8)^{Ba}	2,29 (0,09)^{Cb}	96 (2)^{Aa}
	D	4,6 (0,3)^{Aa}	2,77 (0,12)^{Bb}	10,7 (0,6)^{Aa}	0,50 (0,03)^{Bb}	6,2 (0,8)^{BCa}	6,2 (0,2)^{Cb}	7,7 (0,6)^{BCa}	4,2 (0,3)^{Ba}	85 (2)^{Ab}
TM5B	B	2,3 (0,2)^{Ca}	1,37 (0,09)^{Db}	2,3 (0,2)^{Db}	1,49 (0,06)^{Ca}	3,4 (0,5)^{Dc}	5,8 (0,7)^{Bb}	0,96 (0,09)^{Dc}	0,26 (0,02)^{Cb}	17.6 (0.9)^{Cc}
	GP	0,50 (0,04)^{Cd}	0,86 (0,07)^{Dc}	1,74 (0,09)^{Db}	0,44 (0,03)^{Cc}	0,98 (0,07)^{BCd}	1,67 (0,09)^{Bc}	0^{Ed}	0^{Db}	7.7 (0.7)^{BCd}
	IP	1,36 (0,06)^{Db}	3,2 (0,2)^{Da}	8,5 (0,3)^{BCa}	1,09 (0,07)^{Bb}	6,3 (0,8)^{CDa}	10,29 (1,2)^{Ba}	3,3 (0,5)^{Ca}	3,6 (0,4)^{Ba}	50 (2)^{Ca}
	D	1,00 (0,04)^{Ec}	1,22 (0,09)^{Eb}	8,8 (0,4)^{Ba}	0,96 (0,04)^{Ab}	3,7 (0,3)^{Db}	8,8 (0,7)^{Ba}	1,75 (0,08)^{Db}	3,06 (0,09)^{Ca}	42 (2)^{CDd}
TM10B	B	5,5 (0,3)^{Ba}	3,3 (0,3)^{Bb}	7,5 (0,2)^{Bb}	2,9 (0,3)^{Aa}	6,2 (0,5)^{Ca}	8,4 (0,8)^{Aa}	2,04 (0,09)^{Ca}	0,15 (0,02)^{Db}	45 (3)^{Ac}
	GP	0,94 (0,08)^{Bc}	1,26 (0,09)^{Cd}	2,87 (0,12)^{Bc}	0,72 (0,05)^{Ab}	1,50 (0,09)^{Bb}	1,89 (0,09)^{Ac}	0^{Eb}	0^{Db}	8.97 (0.09)^{Ad}
	IP	1,56 (0,09)^{Db}	3,36 (0,12)B^{Ca}	10,7 (0,9)^{Aa}	1,12 (0,08)^{Bb}	7,8 (1,2)^{BCDa}	8,1 (0,9)^{Ca}	0^{Cb}	3,8 (0,6)^{Ba}	60 (4)^{Ba}
	D	1,57 (0,08)^{Db}	2,61 (0,08)B^{Cc}	10,8 (0,9)^{Aa}	0,92 (0,06)^{Ab}	6,2 (0,9)^{BCa}	5,8 (0,8)^{Cb}	0^{Db}	3,3 (0,3)^{Ca}	47 (3)^{Bb}
AD5B	B	1,22 (0,04)^{Da}	1,94 (0,07)^{Cb}	4,43 (0,12)^{Cc}	1,00 (0,02)^{Da}	8,9 (0,8)^{Ba}	4,0 (0,8)^{Ca}	0,86 (0,04)^{DEd}	0,35 (0,05)^{Ca}	22 (3)^{Cc}
	GP	0,62 (0,03)^{Cb}	2,52 (0,12)^{Ba}	4,30 (0,09)^{Ac}	0,56 (0,04)^{Bc}	3,7 (0,4)^{Ab}	1,73 (0,09)^{ABb}	2,99 (0,09)^{Bc}	0^{Db}	9.0 (0.5)^{Ad}
	IP	0,091 (0,002)^{Ec}	0^{Ed}	8,8 (0,3)^{BCa}	0,74 (0,03)^{Cb}	8,3 (1,2)^{BCa}	0,59 (0,06)^{Ec}	8,8 (1,2)^{Ba}	0^{Db}	50 (3)^{Ca}
	D	0,69 (0,07)^{Eb}	0,83 (0,06)^{Fc}	9,6 (0,4)^{ABa}	0,58 (0,03)^{Bc}	8,7 (0,9)^{Aa}	0,26 (0,02)^{Fc}	6,4 (0,8)^{Cb}	0^{Db}	43 (2)^{BCDb}
AD10B	B	7,4 (0,5)^{Aa}	4,1 (0,3)^{Aa}	10,7 (0,4)^{Aa}	1,82 (0,08)^{Ba}	17 (2)^{Aa}	7,2 (0,9)^{Ab}	0,63 (0,05)^{Eb}	0,91 (0,07)^{Ab}	38 (4)^{Bb}
	GP	0,21 (0,03)^{Dc}	0,95 (0,07)^{Dc}	2,58 (0,12)^{Cc}	0,32 (0,04)^{Dd}	3,8 (0,6)^{Ad}	1,69 (0,03)^{Bc}	1,46 (0,06)^{Cb}	0^{Db}	5.7 (0.8)^{Dc}
	IP	1,36 (0,12)^{Db}	3,85 (0,09)^{Ba}	9,5 (0,3)^{ABb}	1,30 (0,09)^{Ab}	11,7 (1,2)^{Ab}	13,9 (1,2)^{Aa}	8,2 (0,9)^{Ba}	5,8 (0,8)^{Aa}	51 (2)^{Ca}
	D	1,52 (0,07)^{Db}	2,41 (0,08)^{Cb}	9,31 (0,3)^{Bb}	1,01 (0,06)^{Ac}	7,9 (0,9)^{ABc}	11,9 (0,9)^{Aa}	9,31 (1,2)^{Ba}	6,4 (0,8)^{Aa}	38 (3)^{Db}

Letters indicate homogeneous groups established by the ANOVA (p< 0.05) within column for kind of bread fixing state (A-F) and for sample state fixing kind of bread (a-d). CB: Control Bread; P5B: 5 % Pea protein Bread; P10B: 10 % Pea protein Bread; TM5B: 5 % *Tenebrio molitor* Bread; TM10B: 10 % *Tenebrio molitor* Bread; AD5B: 5 % *Alphitobius diaperinus* Bread; AD10B: 10 % *Alphitobius diaperinus* Bread. B, GP, IP and D are Bread, Gastric Phase, Intestinal Phase and Digested sample, respectively.

Implications for gastronomy

Bread may be served in different forms at any meal of the day, eaten as a snack and is even used as an ingredient in other culinary preparations. As a basic food worldwide, bread has come to take on significance beyond mere nutrition, evolving into a fixture in religious rituals, secular cultural life, and language. Alternative protein products are growing in popularity because of consumers trying to change their diets to lead a more sustainable lifestyle. Often consumers are motivated to do so because of the health benefits associated with such eating and drinking habits. Consumers are concerned about the state of the environment and believe that damage done is irreversible. For this reason, alternative sources of protein are an interesting research field for science and gastronomy. Bread can be used as a matrix to introduce alternative proteins in our diet as recognized and accepted food. In this work, alternative sources of proteins as edible insects or pea protein isolates are used to obtain enriched protein bread. As is well known, the nutritional quality of protein depends on its amino acid (AA) content and its physiological application after digestion. AA accessibility varies with protein source, processing methods, and interaction with other components of food like fat and minerals. For this reason, in the present work, AA release from enriched bread with edible insect or pea protein during *in vitro* gastrointestinal digestion is discussed to evaluate their potential use in bakery.

Conflicts of Interest

The authors confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

Author contributions

M.I., P. G. and J. M.: designed the study; M. I., and P.G., conducted the study; P. G. and J. M. performed statistical analysis; M.I. and J. M. wrote the manuscript and had primary responsibility for the final content. All authors have read and approved the final manuscript.