Food Science and Technology 12(1): 81-93, 2024
DOI: 10.13189/fst.2024.120107 http://www.hrpub.org

Antioxidant and Antidiabetic Potential of Fermented Horse Gram and the Sensory Potential as a Reduced-sodium Paste

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Received June 28, 2023; Revised November 22, 2023; Accepted January 26, 2024

Cite This Paper in the Following Citation Styles

(a): *[1] Wan Xin Tan, Li Choo Chong , "Antioxidant and Antidiabetic Potential of Fermented Horse Gram and the Sensory Potential as a Reduced-sodium Paste," Food Science and Technology, Vol. 12, No. 1, pp. 81 - 93, 2024. DOI: 10.13189/fst.2024.120107.*

(b): *Wan Xin Tan, Li Choo Chong (2024). Antioxidant and Antidiabetic Potential of Fermented Horse Gram and the Sensory Potential as a Reduced-sodium Paste. Food Science and Technology, 12(1), 81 - 93. DOI: 10.13189/fst.2024.120107.*

Abstract Hypertension and diabetes are diseases that interrelate which can increase the risk of cardiovascular diseases and one way to combat this issue is to reduce sodium intake. Raw horse gram has demonstrated comparable antioxidative and antidiabetic properties with soybean-fermented products which led to the utilization of horse gram to produce a fermented reduced-sodium functional food. Horse gram was fermented with *Aspergillus oryzae*, and a total of 5 samples containing sodium contents of 9% to 10.5% with a control of 11.7% were prepared. Next, sensory testing was conducted, and the most accepted sample was used for comparison with raw horse gram to determine changes in total phenolic content, antioxidant, and antidiabetic activity. A comparison of the fermented sample with raw horse gram observed that there was an improvement in polyphenol content, ABTS, DPPH activity, α-amylase, and α-glucosidase inhibition activity. The sensory results showed that Sample 1 with 10.5% sodium content had the highest acceptance and overall liking score above 5, suggesting that consumers can accept the sodium reduction. Raw horse gram exhibited good health properties which were enhanced by fermentation processing, and consumers were able to accept a reduced sodium product which encourages further analysis of other health benefits that horse gram can provide and development of other food products.

Keywords Horse Gram, Fermentation, Antioxidant, Antidiabetic

1. Introduction

The World Health Organization [1] reported that cardiovascular diseases (CVDs) are the top causes of death globally where an estimated 17.9 million people died from CVDs in 2019, which represented 32% of all global deaths. Hypertension and diabetes are associated with an increased risk of CVDs, as the interaction could lead to stroke and myocardial infarction development [2]. A study carried out in the United States found that hypertension and diabetes were interrelated [3], as hypertension patients had almost 2.5 times the risk of developing diabetes. Hypertension can be caused by increased blood pressure levels due to overconsumption of sodium [4]. Daily intake of sodium of less than 5g was recommended by the World Health Organization, however, a study done by the Ministry of Health Malaysia found that Malaysians on average consume 7.9g of salt daily [5]. Half of the Malaysian adults that were surveyed added additional seasonings to food at the table which could contribute to the higher salt consumption [5]. This led to the proposal of product reformulation with a reduced sodium content fermented horse gram paste [5].

Fermentation is a food preservation technique that has been around for many centuries and provides new flavors, aromas, and textures, as well as improving the nutritional quality of food by enhancing bioaccessibility and bioavailability, and food safety by inhibiting pathogenic bacteria growth [6,7]. One of the cultivation systems that is used for fermentation is solid-state fermentation (SSF), which uses a solid substrate to enable microbial growth where the free-flowing liquid is absent or near absent [8]. Different microorganisms can be used for food fermentation such as fungus of *Aspergillus* spp., bacteria of *Lactobacillus* spp., and yeasts of *Saccharomyces* spp.; and these foods are commonly present in the daily diet such as soy sauce, miso, yogurt, coffee, tea, bread, and olives [6].

Fermented soy products are commonly seen throughout Asia such as doenjang from Korea, thua nao from Thailand, China's soy sauce, Indonesia's tempeh, and miso from Japan [9]. The processing treatments used for these soy products can induce antioxidant, antidiabetic, anticancer, and anti-inflammatory effects [9]. The traditional Japanese soybean paste of miso is a type of solid-state fermentation where the substrate is typically inoculated with *Aspergillus oryzae* mold to produce koji, which is then added to mashed soybean and salt to undergo further fermentation [10]. The use of *Aspergillus oryzae* to produce koji helps in the development of the distinctive umami flavor as koji fermentation produces glutaminase enzyme which converts glutamine to glutamic acid [10]. The first 5 months of miso fermentation resulted in an intensified flavor and color, which was attributed to the low-temperature Maillard reaction between amino acids and reducing sugars [10]. During the fermentation process, substrate conversion by microorganisms can improve the bioavailability of compounds, decrease antinutritional factors, and improve food safety by detoxifying or removing naturally occurring toxins [6,11]. Miso paste has shown potential health benefits such as antidiabetic effect whereby isoflavonoid glycones produced during fermentation could aid in controlling glucose metabolism; antioxidative effects due to the increase in phenolic compounds; generation of short-chain peptides reduces intestinal inflammation through hydrophobic peptides from miso protein which neutralizes lipopolysaccharides produced by *Escherichia coli*, and antihypertensive effect as proteolytic degradation generates angiotensin-converting enzyme (ACE) inhibitory peptides which aid in blood pressure reduction [12-15].

Horse gram (*Macrotyloma uniflorum*) is a drought, salinity, and heavy metal stress-tolerant legume that is full of nutrients and is encouraged for consumption in developing countries due to its affordability [16]. Horse gram protein provides bioactive peptides that can protect against pathogens, reduce serum triglycerides, and increase lean muscle mass; insoluble dietary fibers that positively affect intestine and colon physiology; and polyunsaturated fatty acids that aid in brain and nervous system development [17]. Research has shown that the horse gram can provide essential amino acids, iron, molybdenum, phosphorus, and the vitamins thiamine and riboflavin, as well as the cotyledon containing a good amount of protein, carbohydrates, and fiber [18].

Horse gram bioactive peptides are rich in polyphenols, phytic acid, flavonoids, and isoflavones which aid in the reduction of oxidative damage to cells, allowing them to have excellent antioxidant and anticarcinogenic activities [17]. Polyphenols especially are compounds of interest due to their great benefits to human health, and horse gram has been ranked as having high phenolic acid content (3.579 ± 0.072 mgGAE/g) [18]. A study comparing polyphenol content of cowpea, horse gram, and chickpea flour extract found that horse gram flour extract showed the highest total polyphenol content of 14.3mgGAE/g [19]. There was a study that observed an association between hypertension and oxidative stress, whereby antioxidant compounds' ability to trap reactive oxygen species could reduce oxidative damage and possibly blood pressure [20]. Insulin resistance and impaired insulin secretion have been associated with oxidative stress, so improving antioxidant activity could have a positive impact on the treatment of diabetes [21].

Aside from the antioxidant property affecting diabetes, horse gram has also demonstrated antidiabetic properties as it induces low postprandial glucose response due to the slow-digesting starch present [22]. The enzymes α-amylase and α-glucosidase help to break down dietary polysaccharides into monosaccharides to allow digestion in the small intestine, causing the rise in postprandial blood glucose [23]. Inhibition of these enzymes could help with the control of diabetes since starch digestion would be slowed down, leading to a decrease in glucose absorption rate which will then slow down postprandial blood glucose increase [23]. Research has observed potent intestinal α-glucosidase and α-amylase inhibitors that were isolated from horse gram-inhibited mice and human salivary α-amylase which can strengthen claims of antidiabetic properties [17,22].

The health benefits of horse gram have been increasingly studied which also encourages more research on producing a functional food product from the legume [18]. Horse gram fermented with *Penicillium camemberti* known as Kaulath was compared with unfermented horse gram, and Kaulath showed an increase in free radical scavenging and α-glucosidase activity indicating its potential as a functional ingredient for consumption [24]. Joshi and Awasthi [25] added horse gram flour to whole wheat flour and were able to develop highly acceptable biscuits that had an improved nutritional value. Another study used germinated horse gram to fortify bread flour which increased protein, fiber, total polyphenol content, and antioxidant activity indicating that this method of processing horse gram can be used as a food ingredient [26]. This study aims to apply a process similar to that of miso fermentation by fermenting horse gram using koji made with *Aspergillus oryzae* mold to improve the nutritional quality of horse gram to increase its potential as a

functional food product while ensuring that the reduction of sodium as compared to commercial products can still be accepted by consumers.

2. Materials and Methods

Preparation of paste was done using raw horse gram, rice koji (rice fermented with *Aspergillus oryzae*), and salt which were all purchased from local retailers in Malaysia. Alcalase enzyme, Folin-Ciocalteu reagent, gallic acid, ABTS tablets, DPPH powder, α-amylase enzyme, DNS (3,5-dinitrosalicyclic acid), α-glucosidase enzyme and NGP (4-nitrophenyl-α-D-glucopyranoside) were all purchased from Sigma-Aldrich Chemicals (St Louis, MO, USA).

2.1. Preparation of Fermented Horse Gram Paste

To develop the product, the methods of Inoue et al. were followed but with modifications [27]. 500g of horse gram was rinsed and soaked overnight, then rinsed again before boiling for 2 hours until horse gram can be crushed between fingers. The water used to soak horse gram was strained using a sieve, then a blender was used to homogenize the horse gram and it was set aside to cool down. Glass jars were boiled and dried in an oven at 120˚C for 15 minutes to ensure they were dry and sterilized before horse gram was fermented in them. Mashed horse gram, salt, and rice koji were mixed and tightly packed into the glass jar. A total of 5 samples of horse gram pastes with salt percentages of 11.7% (control), 10.5%, 10%, 9.5% and 9% were prepared. The Guide to Nutrition Labelling and Claims was used as a reference to calculate salt reduction [28]. The samples were left to ferment in an enclosed area for 6 months at room temperature, and the samples were inspected for unusual growth and cleaned with a vinegar-soaked cloth to prevent unwanted mold growth.

As a comparison was being made to a commercial product, the Guide to Nutrition Labelling and Claims was used to determine the amount of sodium that could be reduced when fermenting the horse gram paste [28]. The comparative claim could only be made when the following criteria were met:

1) When foods are being compared, there must be at least a 10% difference in micronutrient content.
2) For a product to be claimed as “reduced”, Appendix 7 or 8 of the Guide to Nutrition Labeling and Claims states the value needed for a minimum absolute difference in energy value or nutrient content [28]. The value for sodium content is 0.12g per 100g.

Based on the criteria stated, the following equations were used to calculate the appropriate reduction of salt added during fermentation:

$$(a-b)/a \times 100\%$$

a = Sodium content of commercial product

b = Sodium content of the sample

$$(c \times 2.54)/1000$$

c = difference in sodium content between commercial product and sample

2.2. Sample Hydrolysate Preparation

After 6 months of fermentation, the horse gram paste was analyzed by preparing hydrolysates of the paste using the method developed by Bhaskar et al. [29]. The sample was mixed with distilled water in a ratio of 1/10 (w/v) in a flask, then adjusted to pH 8 with 0.1M NaOH. 1g of Alcalase enzyme was then added and incubated at 55˚C for 3 hours. The pH of the sample was maintained throughout the incubation period. After that, the sample was heated at 90˚C for 10 minutes for enzyme inactivation, and centrifuged at 10,000g for 20 minutes to obtain supernatant. Fermented horse gram hydrolysate was placed in a -20˚C freezer for analysis.

2.3. Total Polyphenol Content (TPC)

The method of Lee et al was used to determine the total polyphenol content, but with modifications [30]. 0.1ml of sample pre-diluted at a ratio of 1:1 was added to a boiling tube containing 1.9ml of deionized water and 1ml of Folin Ciocalteu reagent. The samples were left to stand in the dark for 8 minutes, then 5ml of 20% Na_2CO_3 was added and placed in a boiling water bath for 1 minute. Samples were cooled in darkness and absorbance was then measured at 750nm. All samples were prepared in triplicates. Gallic acid was used to prepare the standard curve and results were reported as mg of gallic acid equivalent/g horse gram.

2.4. Antioxidant Activity

2.4.1. ABTS Assay

ABTS activity was measured using the method suggested by Re et al. [31]. A concentration of 7mM of ABTS reagent was prepared and 2.45mM of potassium peroxydisulfate was added and left in an enclosed area away from light overnight. 5mL of ABTS+ reagent and 54mL of deionized water were mixed in a beaker to obtain an absorbance value of 0.7 (±0.02) at 734nm. The diluted reagent was loaded at a volume of 90 µL into the well of 96 – well microplate and 10 µL of the sample was added to each well with the reagent. Absorbance was measured at 734nm after 6 minutes. All samples were prepared in triplicates. The standard curve was constructed using Trolox as a standard solution.

The results were calculated using the formula:

$$\text{ABTS inhibition (\%)} = (Ac - As)/As \times 100\%$$

Ac = Control absorbance
As = Sample absorbance

2.4.2. DPPH Assay

The Brand–Williams et al. method with modifications was used to determine DPPH free radical scavenging activity [32]. 1.26mM of DPPH reagent was prepared with ethanol and the absorbance of the reagent was adjusted to 1.08 – 1.12 at a wavelength of 517nm. 280 µL of reagent, 10 µL of ethanol, and 10 µL of the sample were mixed in a tin foil-wrapped centrifuge tube. The tubes were left away from light for 24 hours and absorbance was read at 517nm. All samples were prepared in triplicates. The calibration curve was constructed using Trolox with concentrations of 0.1 – 0.5 Mm. Control was prepared without the addition of a sample. Results were expressed as percentages in the reduction of DPPH free radicals.

$$\text{\% Inhibition: } (Ac - As)/Ac \times 100\%$$

Ac = Control
As = Sample

2.5. Antidiabetic Activity

2.5.1. α-amylase Inhibition Activity

To determine α-amylase inhibition activity the method suggested by Kwon et al. was used but with modifications [33]. A 20mM sodium phosphate buffer containing porcine α-amylase (pH 6.9) with 4U/mL concentration was prepared. Next 250 µL of sample was added and incubated at 37˚C for 10 minutes. 1% of starch solution in the same buffer was added and incubated for another 10 minutes. 96mM 3,5-dinitro salicylic acid (DNS) mixed with 2N NaOH was added and boiled for 5 minutes. The control replaced the sample with methanol. Samples were removed from heat and cooled before measuring absorbance at 540nm against a blank of PBS. All samples were prepared in triplicates.

$$\text{\% α-amylase inhibition} = (Ac - As)/Ac \times 100\%$$

Ac = Control absorbance
As = Sample absorbance

2.5.2. α-glucosidase Inhibition Activity

The method suggested by Kwon et al. was used with modifications to determine α-glucosidase inhibition activity [34]. 250µL of the sample was added to 500 µL α-glucosidase with sodium phosphate buffer (0.1M) and incubated for 10 minutes at 37˚C. 500µL of 5mM 4-nitrophenyl-α-D-glucopyranoside (NGP) was added to the solution and absorbance was immediately measured. It was then placed in an incubator for 15 minutes at 37˚C. 405nm was used to measure absorbance and control was prepared by substituting the sample with methanol. All samples were prepared in triplicates.

$$\text{\% α- Glucosidase inhibition} = (\Delta Ac - \Delta As)/\Delta Ac \times 100\%$$

ΔAc = Difference between initial and final incubation absorbance of control

ΔAs = Difference between initial and final incubation absorbance of sample

2.6. Sensory Analysis

Commercial miso paste Marukome boy awase was prepared along with the fermented horse gram paste of Sample 1 (10.5% salt content), Sample 2 (10% salt content), Sample 3 (9.5% salt content), Sample 4 (9% salt content), and Control sample (11.7% salt content) to compare the reaction of the panel to the attributes. The samples were consumed as soup, and it was prepared by adding the sample to boiling water and allowing it to boil for 5 minutes. The soup was cooled and 20ml was placed in paper cups, and before serving small pieces of tofu and a pinch of pepper was added to the soup. The amount of sample paste added to boiling water was based on a ratio of 1:10 (w/v). The first part of the questionnaire was a 9-point hedonic scale test where panelists ranked the degree of liking of each of the attributes of individual samples which were appearance, aroma, taste, saltiness, and also the degree of liking the samples overall [35].

The second part of the questionnaire had the panel comparing all the samples and ranking them in order from '1-not acceptable' to '6-most acceptable' based on salt taste intensity and sample acceptability. All samples appeared an equal number of times and each sample had random 3-digit identifiers. Panels were given mineral water to cleanse their palates between samples. The requirements of the panel were as follows:

1) Aged between 18 – 50
2) No pre-existing health conditions
3) Able to consume non-halal food
4) Understand what miso paste is and know the taste
5) Has no issues with distinguishing different tastes in liquids

The data obtained from the questionnaire was used to decide which sample had the highest overall acceptability by panelists and was used to determine the antioxidant and antidiabetic properties.

2.7. Statistical Analysis

SPSS V.20 was used to analyze all data and $p < 0.05$ was used to define the significant difference.

3. Results

To determine the total phenolic content of raw and fermented horse gram, gallic acid was used to prepare the

standard curve and the values were expressed as gallic acid equivalents. From the results shown in Table 1, it was observed that the TPC of fermented horse gram had increased in comparison to raw horse gram.

Table 1. TPC of raw and fermented horse gram

	Raw horse gram	Fermented horse gram
Total phenolic content (mgGAE/g)	4.775 ± 0.177	5.635 ± 0.556

*The values represent the mean of TPC ± standard deviation.

The comparison between raw and fermented horse gram showed an increase in percentage inhibition of both ABTS and DPPH assay (Table 2). As ABTS assay percentage inhibition was already high, there was only a slight increase in the percentage inhibition of fermented horse gram. DPPH assay of fermented horse gram showed a higher increase in percentage inhibition as raw horse gram percentage inhibition was relatively lower.

Table 2. ABTS and DPPH assay results of raw and fermented horse gram

	Raw horse gram	Fermented horse gram
ABTS (%inhibition)	93.87 ± 0.167	94.48 ± 0.145
DPPH (%inhibition)	49.71 ± 2.401	64.85 ± 0.805

*The values represent the mean of percentage inhibition ± standard deviation.

As seen from Table 3, raw horse gram showed a lower percentage inhibition of α-amylase inhibition compared to α-glucosidase inhibition. A comparison of raw horse gram and fermented horse gram α-amylase and α-glucosidase inhibition showed that there was a percentage increase for both inhibition activities. Although fermented horse gram had an increase in both α-amylase and α-glucosidase inhibition, it was observed that α-glucosidase inhibition had a higher percentage increase compared to that of α-amylase inhibition.

Table 3. α-amylase and α-glucosidase inhibition activity of raw and fermented horse gram

	Raw horse gram	Fermented horse gram
α-amylase inhibition (% inhibition)	61.40 ± 1.381	65.94 ± 1.155
α-glucosidase inhibition (% inhibition)	81.34 ± 0.720	98.08 ± 1.890

*The values represent the mean of percentage inhibition ± standard deviation.

Table 4 shows that the panelists preferred the smell of the commercial sample as it had the highest score of 6.48. Although overall liking of smell for all samples was not significantly different, Sample 1 was the only sample to have a moderate score of 5.21 and the other samples were below a score of 5. Panelists also preferred the taste of the commercial sample as there was a significant difference in the liking of taste score of 6.63. Sample 1 had a significant difference in liking of taste score compared to Sample 4, however comparison with Control, Sample 2, and Sample 3 showed no significant difference. However, as was seen with the rating of the attribute of liking of smell, Sample 1 scored the highest for liking of taste with 5.33, while all the other samples had a mean score below 5. The rating of the overall acceptability of samples showed that the commercial sample had the highest score (6.59) which was significantly different from the other samples. There was no significant difference between the samples, however, only Sample 1 and Sample 2 had an overall acceptability rating score above 5. The commercial sample had the highest overall acceptability, while Sample 1, Sample 2, and Sample 3 did not show significant differences in ranking; and Control and Sample 4 had significantly lower overall acceptability rankings.

Table 4. Results of Sensory Analysis

Sample (salt %) (3-digit identifiers) / Attribute rating	Commercial seasoning (11.7%) (251)	Control (11.7%) (748)	Sample 1 (10.5%) (920)	Sample 2 (10%) (563)	Sample 3 (9.5%) (807)	Sample 4 (9%) (372)
Liking of appearance*a*d	6.427 ±0.251^{f}	5.253 ±0.237^{g}	5.56 ±0.243^{g}	5.240 ±0.223^{g}	4.933 ±0.230^{g}	4.787 ±0.236^{g}
Liking of smell*a*d	6.480 ±2.030^{f}	4.613 ±1.958^{g}	5.213 ±1.812^{g}	4.813 ±1.894^{g}	4.813 ±2.120^{g}	4.720 ±2.083^{g}
Liking of taste*a*d	6.627 ±2.229^{f}	4.427 ±1.876^{g}	5.333 ±1.78^{g}	4.987 ±1.856^{g}	4.600 ±2.047^{g}	4.307 ±2.143^{h}
Liking of saltiness*a*d	5.560 ±2.389^{f}	4.787 ±1.984^{f}	5.227 ±1.820^{f}	5.027 ±1.938^{f}	4.907 ±1.932^{f}	4.933 ±2.095^{f}
Individual acceptability*a*d	6.587 ±2.014^{f}	4.707 ±1.807^{g}	5.253 ±1.952^{g}	5.107 ±1.914^{g}	4.800 ±1.938^{g}	4.613 ±1.944^{g}
Salt intensity*c*e	4.000 IQR 4^{i}	4.000 IQR 3^{i}	3.000 IQR 2^{j}	3.000 IQR 2^{j}	4.000 IQR 3^{i}	4.000 IQR 3^{i}
Overall acceptability*b*d	5.171 ± 1.544^{f}	2.895 ±1.466^{h}	3.697 ±1.617^{g}	3.184 ±1.449^{g}	3.158 ±1.396^{g}	2.882 ±1.625^{h}

*a Ranked individually based on a scale of ‘1 – Dislike extremely’ to ‘9 – Like extremely’

*b Ranked together based on a scale of ‘1 – Least acceptable’ to ‘6 – Most acceptable’

*c Ranked together based on a scale of ‘1 – Least intense’ to ‘6 – Most intense’

*d Mean results and standard deviation reported

*e Median results and interquartile range (IQR) reported

fgh Within a row means without a common superscript differ ($p < 0.05$)

ij Within a row, medians without a common superscript differ ($p < 0.05$)

4. Discussion

Biochemical changes are expected to happen over the course of the fermentation period which can change the nutritive and anti-nutritive content affecting the bioaccessibility and bioavailability of nutrients [36]. TPC has been shown to increase through fermentation processes and legume phenolic content has been determined to be 0.325 – 6.378 mgGAE (gallic acid equivalent)/g, and with a TPC of 3.579 ± 0.072 mgGAE/g horse gram is classified as part of the high phenolic acid group [18]. The TPC of raw horse gram hydrolysate was higher than previously mentioned, however, Ojha et al. [37] reported similar values for whole horse gram (4.6 ± 0.30 mgGAE/g). While TPC cannot be directly compared between studies, similarities were observed between values obtained for TPC of cooked horse gram sprouts fermented with lactic acid bacteria of two different strains which were 5.80 ± 0.20 and 5.41 ± 0.16 mgGAE/g and values obtained from fermented horse gram of this research [12]. Fermented horse gram with rice koji also showed superior TPC compared to soybean (3.18mgGAE/g DW), chickpea (1.47mgGAE/g DW), black bean (3.31mgGAE/g DW), and mung bean (3.81mgGAE/g DW) fermented with *Cordyceps militaris* [38]. The increase in TPC during fermentation is attributed to the metabolic activities of the microflora which suggests that the proteolytic activities of the microorganism *Aspergillus oryzae* in the koji ingredient catalyzed the release of total phenolics leading to the increase in TPC shown above [39].

The fermentation of horse gram had an increase in antioxidant activity compared to raw horse gram as the TPC of fermented horse gram was higher than raw horse gram. DPPH assay showed an increase in percentage inhibition when comparing raw and fermented horse gram, whereas Ojha et al. [37] also reported a similar trend as DPPH % inhibition increased from 52.68% to 59.92% when comparing raw horse gram and fermented horse gram. Horse gram that was fermented with *Penicillium camemberti* observed the potential of horse gram as a functional food and a higher DPPH inhibition activity was found in the horse gram fermented with *Penicillium camemberti* (26%) compared to unfermented horse gram (19.3%) [24]. Singh et al. [40] suggested bioactive components of isoflavones, saponins, and phenolic compounds may be produced during fermentation as the fermentation of soy milk with five test strains of lactobacilli all produced higher ABTS and DPPH activity than unfermented soy milk, where radical scavenging activity of ABTS increased from 20%-40% to 63%-70% and DPPH activity increased from 20%-30% to 63%-64%, results which were also observed with fermented horse gram. Ojha et al. [37] proposed that the increase in antioxidant activity was due to the initiation of polyphenol and flavonoid synthesis during fermentation.

Phenolic compounds provide good antioxidant activity due to their ability to donate hydrogen atoms which deactivate free radicals, and the increase of phenolic content contributes to the increase in antioxidant activity [41]. During fermentation starter cultures used also contribute to the increased antioxidant activity as the proteolytic enzymes could hydrolyze glycosidic bonds and release free phenolics, while the increase in acidity liberates bound free flavonoids [25]. This can be observed from Table 1 where fermented horse gram had an increase in TPC compared to raw horse gram and Table 2 also showed an increase in antioxidant activity when comparing raw and fermented horse gram. A similar observation was made by a study comparing chungkookjang (Korean fermented soybean paste) with unfermented steamed soybeans and found that chungkookjang had stronger antioxidant activity due to fermentation increasing total polyphenol contents and that of isoflavones [42]. Basmati rice and the bean seim were fermented with fungal strains of *Aspergillus oryzae* and *Aspergillus awamori* and it was observed that as TPC values increased over the incubation period, ABTS and DPPH activity increased as well [43].

Reactive oxygen species (ROS) can cause oxidative stress which leads to cell membrane disintegration, and this imbalance of ROS and antioxidant protection induces diabetes mellitus [44]. Reduction of oxidative stress helps to manage diabetes which has increased interest in natural antioxidants from plant compounds [44]. Prasad & Singh [18] discussed horse gram's nutritional properties by stating its richness in bioactive compounds of polyphenols, proteins, phytic acid, flavonoids, and isoflavones; particularly horse gram seed coat exhibiting good antioxidant activity as it contains considerable amounts of phenolic acids, flavonoids, and tannins [17]. As seen in Table 2 horse gram demonstrated high antioxidant activity, which was also demonstrated in unfermented (40.1%) and fermented pigeon peas (56.2%) [45]. Biochemical processes are initiated during fermentation such as phytase activity and polyphenol and flavonoid synthesis, thus contributing to the increased antioxidant activity [37].

Hypertension and diabetes are interrelated as seen in type 2 diabetes where insulin resistance that occurs results in dyslipidemia, elevated inflammatory markers, and production of reactive oxygen species that deplete vascular endothelial nitric oxide which causes an increase in blood pressure [46]. It was observed that polyphenolic fractions from plants inhibit α-amylase and α-glucosidase enzyme activity leading to the rising interest in the antidiabetic properties of horse gram due to its potential α-amylase and α-glucosidase enzyme inhibitors [17]. Table 3 showed that fermentation can improve the antidiabetic properties of horse gram as there was an increase in percentage inhibition of both α-amylase and α-glucosidase inhibition compared to raw horse gram. A similar trend of increase in α-amylase inhibition activity was observed in lentils fermented with *Aspergillus oryzae*, a similar fungal species that was used for this horse gram fermentation, where the fermented lentils increased α-amylase inhibition activity from 71% to 75% [47]. Fermented and unfermented black

rice bran was added to analog rice to compare its effects, and a higher α-amylase inhibition activity (63.16%-65.51%) was observed in analog rice with fermented black rice bran compared to that of unfermented black rice bran (53.03%-55.20%) [48]. Previously mentioned horse gram fermented with *Penicillium camemberti* also observed an increase in α-glucosidase inhibition activity between raw (9.3%) and fermented horse gram (10.8%) [24]. Lee et al. [49] fermented soybeans with *Tricholoma matsutake* and observed a high increase in α-glucosidase inhibition which was from 11.8% to 84.9%. The side effects of using commercially available inhibitors such as acarbose or voglibose result in abnormal distention, flatulence, and diarrhea, which could be due to abnormal bacterial fermentation of undigested carbohydrates in the colon from excessive inhibition of pancreatic α-amylase [33]. Therefore, from the results obtained in Table 3, horse gram has shown potential as a solution to this issue due to its strong α-glucosidase inhibitory activity and lower α-amylase inhibitory activity.

Based on the results from Table 4 the liking of appearance, smell, saltiness, and individual acceptability of all samples did not have a significant difference. Sample 1 had a significant difference in liking of taste score compared to Sample 4, but there was no significant difference with Control, Sample 2, and Sample 3. When reducing the sodium content of fermented foods, the metabolic activities of microorganisms that contribute to the flavor profile are affected which changes the flavor of the product [50], and the salt content reduction of 1.5% between Sample 1 and Sample 4 was sufficient to produce a significant difference in liking of taste score. A similar finding was reported by Liu et al. [51] as soy sauce prepared with four different salt contents of 8%, 12%, 16%, and 20% showed that 16% scored the highest while lower salt content of 8% had an undesirable smell and a decrease umami flavor. Fermentation of koji and broad beans to produce doubanjiang also reported a decreased score for the flavor profile of doubanjiang when the salt content was decreased [52]. From the results obtained in Table 4, there was a trend of decreasing rating scores as salt content decreased, which resulted in Sample 4 of the lowest salt content having the significantly lowest overall acceptability. Similarly, doenjang a Korean traditional fermented soybean pastes prepared using salt contents of 8%, 12%, 16%, and 20%, observed that the 8% paste had the lowest consumer acceptance rating as attributes of appearance, odor, taste, and mouthfeel were less developed over the aging period [53].

Miso paste is commonly used in Japanese cuisine and the flavor profile is described as savory or umami, however, it has been gaining popularity among chefs and home cooks where it is incorporated to produce savory and sweet foods [10]. This has also allowed a larger audience to become familiar with the taste of miso paste, so during the sensory analysis, the panel could have some sense of familiarity when sampling the soups to provide feedback. Table 4 showed that the commercial sample had the best rating overall, which could be expected as it is a marketed good. The liking of saltiness attribute did not have a significant difference between samples which could be due to the salt content difference of 0.5% not being enough to produce a distinguished salty taste. The umami taste in the horse gram paste is developed during the fermentation process when hydrolytic enzymes in the koji hydrolyze the protein into peptides containing amino acids of glutamic acid, aspartic acid, and proline [54]. This could have also contributed to the insignificant difference in saltiness liking as Kremer et al. [55] suggested that foods containing natural umami substances could have a decrease in salt content without affecting consumer acceptance.

5. Conclusions

The good antidiabetic activity that fermented horse gram shows could play a part in managing diabetes which will help to reduce hypertension risk. In vitro assessment of fermented horse gram product has shown potential nutritional properties such as the good antioxidant activity of high phenolic content, ABTS and DPPH inhibition, and good antidiabetic activity of α-amylase and α-glucosidase inhibition activity. Through the sensory analysis, consumers have also shown an acceptance of a reduced sodium product made from fermented horse gram. This encourages further research into the production of fermented horse gram into a commercial good that also provides health benefits and improves crop diversity. Future studies can include research on antihypertensive and antihypercholesterolemic properties and *in vivo* assessments to determine whether the nutritional properties mentioned are just as effective after undergoing the gastrointestinal digestion process.

Acknowledgements

The authors gratefully acknowledge the financial support received under the Fundamental Research Grant Scheme (FRGS/1/2019/SKK06/TAYLOR/02/1) from the Ministry of Higher Education Malaysia (MOHE). The authors also thank Taylor's University Food Security & Nutrition Impact Lab for their help. The authors are thankful to the volunteers who participated in providing sensory data for the study.

Conflict of Interest

There is no conflict of interest.

Appendix

Questionnaire for sensory analysis of fermented horse gram paste
Age:
Gender: M / F

A. 9-point Hedonic Scale

Instructions:

1. Please use water provided to rinse mouth before starting and in between sample tasting.
2. Please write down the 3-digit code of sample tasted accordingly.
3. Please circle the number that best describes each sample.

Table 5. Panelist rating and liking score

Panelist Rating	Liking Score
Like Extremely	9
Like Very Much	8
Like Moderately	7
Like Slightly	6
Neither Like nor Dislike	5
Dislike Slightly	4
Dislike Moderately	3
Dislike Very Much	2
Dislike Extremely	1

Table 6. Liking score of attributes of sample

Attributes	Liking Score								
Appearance:	1	2	3	4	5	6	7	8	9
Smell/Aroma:	1	2	3	4	5	6	7	8	9
Taste:	1	2	3	4	5	6	7	8	9
Saltiness:	1	2	3	4	5	6	7	8	9
Overall Acceptability:	1	2	3	4	5	6	7	8	9
Overall Acceptability:	1	2	3	4	5	6	7	8	9

Sample Code:_____

B. Sample Ranking Test

1. Please rank the **intensity of the salty taste** of samples from '1- not intense' to '6-most intense'. (Please write the numbers clearly, thank you).

Table 7. Ranking score of intensity of salty taste

Ranking	
Most intense	6
Very intense	5
Moderately intense	4
Slightly intense	3
Least intense	2
Not intense	1

Sample code		**Ranking**
i.	920	
ii.	563	
iii.	807	
iv.	372	
v.	748	
vi.	251	

2. Please rank the **acceptability of samples** from '1-not acceptable' to '6-most acceptable'. (Please write the numbers clearly, thank you)

Table 8. Ranking score of acceptability of samples

Ranking	
Most acceptable	6
Very acceptable	5
Moderately acceptable	4
Slightly acceptable	3
Least acceptable	2
Not acceptable	1

Sample code		**Ranking**
i.	920	
ii.	563	
iii.	807	
iv.	372	
v.	748	
vi.	251	

REFERENCES

[1] "Cardiovascular diseases (CVDs)." World Health Organization. https://www.who.int/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds) (accessed Sept. 2, 2022).

[2] M. Ohishi. Hypertension with diabetes mellitus: physiology and pathology, Hypertension Research, Vol.41, 389-393, 2018, DOI: 10.1038/s41440-018-0034-4.

[3] B.M.Y. Cheung, C. Li. Diabetes and hypertension: is there a common metabolic pathway?. Current Atherosclerosis Reports, Vol.14, No.2, 160-166, 2012, DOI: 10.1007/s11883-012-0227-2.

[4] A. Grillo, L. Salvi, P. Coruzzi, P. Salvi, G. Parati. Sodium intake and hypertension, Nutrients, Vol.11, No.9, 2019, DOI: 10.3390/nu11091970.

[5] Ministry of Health Malaysia, Salt Reduction Strategy to Prevent and Control NCD for Malaysia 2021-2025, Ministry of Health Malaysia, Malaysia, 2021. Online available from https://www.moh.gov.my/moh/resources/Penerbitan/Rujukan/NCD/Garam/Salt_Reduction_Strategy_To_Prevent_And_Control_NCD_For_Malaysia_2021-2025_26082021.pdf

[6] S. Garrido-Galand, A. Asensio-Grau, J. Calvo-Lerma, A. Heredia, A. Andrés. The potential of fermentation on nutritional and technological improvement of cereal and legume flours: A review, Food Research International, Vol.145, 2021, DOI: 10.1016/j.foodres.2021.110398.

[7] S. G. Nkhata, E. Ayua, E. H. Kamau, J. B. Shingiro. Fermentation and germination improve nutritional value of cereals and legumes through activation of endogenous enzymes, Food Science & Nutrition, Vol.6, No.8, 2446-2458, 2018, DOI: 10.1002/fsn3.846.

[8] T. B. Dey, S. Chakraborty, K. K. Jain, A. Sharma, R. C. Kuhad. Antioxidant phenolics and their microbial production by submerged and solid state fermentation process: A review, Trends in Food Science & Technology, Vol.53, 60-74, 2016, DOI: 10.1016/j.tifs.2016.04.007.

[9] M. Jayachandran, B. Xu. An insight into the health benefits of fermented soy products, Food Chemistry, Vol.271, 362-371, 2019, DOI: 10.1016/j.foodchem.2018.07.158.

[10] J. G. Allwood, L. T. Wakeling, D. C. Bean. Fermentation and the microbial community of Japanese koji and miso: A review, Concise Reviews & Hypotheses in Food Science, Vol.86. No.6, 2194-2207, 2021, DOI: 10.1111/1750-3841.15773.

[11] H. A. Sakandar, Y. Chen, C. Peng, X. Chen, M. Imran, H. Zhang. Impact of fermentation on antinutritional factors and protein degradation of legume seeds: A review, Food Reviews International, Vol.39, No.3, 1227-1249, 2021, DOI: 10.1080/87559129.2021.1931300.

[12] D. Y. Kwon, J. W. Daily III, H. K. J. Kim, S. Park. Antidiabetic effects of fermented soybean products on type 2 diabetes, Nutritional Research, Vol.30. No.1, 1-13, 2010, DOI: 10.1016/j.nutres.2009.11.004.

[13] L. Chen, R. L. Madl, P.V. Vadlani, L. Li, W. Wang. Soybean: Bio-active compounds, InTech, United Kingdom, 2013, DOI: 10.5772/52993.

[14] H. Sasaki, P. T. N. Diep, M. Nishikawa, M. Kanauchi. Lipopolysaccharide neutralizing protein in Miso, Japanese fermented soybean paste, Journal of Food Science, Vol.85, No.8, 2498-2505, 2020, DOI: 10.1111/1750-3841.15315.

[15] S. Sanjukta, A. K. Rai. Production of bioactive peptides during soybean fermentation and their potential health benefits, Trends in Food Science & Technology, Vol.50, 1-10, 2016, DOI: 10.1016/j.tifs.2016.01.010.

[16] J. Bhardwaj, S. K. Yadav. "Drought Stress Tolerant Horse Gram for Sustainable Agriculture," in Sustainable Agriculture Reviews, 15th ed, Springer, America, 2014. DOI: 10.1007/978-3-319-09132-7_7.

[17] J. P. Aditya, A. Bhartiya, R. K. Chahota, D. Joshi, N. Chandra, L. Kant, A. Pattanayak. Ancient orphan legume horse gram: a potential food and forage crop of future, Planta, Vol.250, 891-909, 2019, DOI: 10.1007/s00425-019-03184-5.

[18] S.K. Prasad, M. K. Singh. Horse gram- an underutilized nutraceutical pulse crop: A review, Journal of Food Science and Technology, Vol.52, No.5, 2489–2499, 2015, DOI: 10.1007/s13197-014-1312-z.

[19] Y. N. Sreerama, V. B. Sashikala, V. M. Pratape. Phenolic compounds in cowpea and horse gram flours in comparison to chickpea flour: Evaluation of their antioxidant and enzyme inhibitory properties associated with hyperglycemia and hypertension, Food Chemistry, Vol.133, No.1, 156 – 162, 2012, DOI: 10.1016/j.foodchem.2012.01.011.

[20] A. Baradaran, H. Nasri, M. Rafieian-Kopaei. Oxidative stress and hypertension: Possibility of hypertension therapy with antioxidants, Journal of Research in Medical Sciences, Vol.19, No.4, 358-367, 2014, PMCID: PMC4115353, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4115353/.

[21] D. Pellegrino. Antioxidants and cardiovascular risk factors. Diseases. Vol.4, No.1, 2016, DOI: 10.3390/diseases4010011.

[22] S. P. Kaundal, A. Sharma, R. Kumar, V. Kumar, R. Kumar. Exploration of medicinal importance of an underutilized legume crop, Macrotyloma uniflorum (Lam.) verdc. (horse gram): A review, International Journal of Pharmaceutical Sciences and Research, Vol.10, No.7, 3178-3186, 2019, DOI: 10.13040/IJPSR.0975-8232.10(7).3178-86.

[23] A. B. Shori. Screening of antidiabetic and antioxidant activities of medicinal plant, Journal of Integrative Medicine, Vol.13, No.5, 297-305, 2015, DOI: 10.1016/S2095-4964(15)60193-5.

[24] M. Dwidevi, K. Y. Vasantha, Y. N. Sreerama, D. J. Haware, R. P. Singh, A. P. Sattur. Kaulath, a new fungal fermented food from horse gram, Journal of Food Science and Technology, Vol.52, No.12, 8371-8376, 2015, DOI: 10.1007/s13197-015-1887-z.

[25] H. Joshi, P. Awasthi. Evaluation of physical properties and sensory attributes of biscuits developed from whole wheat flour supplemented with horse gram flour, Journal of Pharmacognosy and Phytochemistry, Vol.9, No.5, 1652-1656, 2020, [Online] Available at:

https://www.phytojournal.com/archives/2020/vol9issue5/PartW/9-5-274-806.pdf.

[26] K. Moktan, P. Ojha. Quality evaluation of physical properties, antinutritional factors, and antioxidant activity of bread fortified with germinated horse gram (Dolichus uniflorus) flour, Food Science & Nutrition, Vol.4, No.5, 766 – 771, 2016, DOI: 10.1002/fsn3.342.

[27] K. Inoue, T. Gotou, H. Kitajima, S. Mizuno, T. Nakazawa, N. Tamamoto. Release of antihypertensive peptides in miso paste during its fermentation, by the addition of casein, Journal of Bioscience and Bioengineering, Vol.108, No.2, 111-115, 2009, DOI: 10.1016/j.jbiosc.2009.03.007.

[28] E. S. Tee., "Guide To Nutrition Labelling and Claims (as of December 2010)", Ministry of Health Malaysia, https://extranet.who.int/nutrition/gina/sites/default/filesstore/MYS%202010%20Guide%20to%20Nutrition%20Labelling%20and%20Claims.pdf (accessed Aug. 7, 2022).

[29] B. Bhaskar, L. Ananthanarayan, S. N. Jamdar. Effect of enzymatic hydrolysis on the functional, antioxidant, and angiotensin I-converting enzyme (ACE) inhibitory properties of whole horse gram flour, Food Science and Biotechnology, Vol.28, No.1, 43-52, 2019, DOI: 10.1007/s10068-018-0440-z.

[30] I. H. Lee, Y. H. Hung, C. C. Chou. (2008). Solid-state fermentation with fungi to enhance the antioxidative activity, total phenolic and anthocyanin contents of black bean, International Journal of Food Microbiology, Vol.121, No.2, 150-156, 2008, DOI: 10.1016/j.ijfoodmicro.2007.09.008.

[31] R. Re, N. Pellegrini, A. Proteggente, A. Pannala, M. Yang, C. Rice-Evans. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay, Free Radical Biology & Medicine, Vol.26, No.9-10, 1231-1237, 1999, DOI: 10.1016/s0891-5849(98)00315-3.

[32] W. Brand-Williams, M. E. Cuvelier, C. Berset. Use of a free radical method to evaluate antioxidant activity, LWT-Food Science and Technology, Vol.28, No.1, 25-30, 1995, DOI: 10.1016/S0023-6438(95)80008-5.

[33] Y. I. Kwon, E. Apostolidis, K. Shetty. Anti-diabetes functionality of Kefir culture-mediated fermented soymilk supplemented with Rhodiola extracts, Food Biotechnology, Vol.20, No.1, 13-29, 2007, DOI: 10.1080/08905430500522055.

[34] Y. I. Kwon, E. Apostolidis, K. Shetty. In vitro studies of eggplant (Solanum melongena) phenolics as inhibitors of key enzymes relevant for type 2 diabetes and hypertension, Bioresource Technology, Vol.99, No.8, 2981-2988, 2008, DOI: 10.1016/j.biortech.2007.06.035.

[35] L. Nicolas, C. Marquilly, M. O'Mahony. The 9-point hedonic scale: Are words and numbers compatible?, Food Quality and Preference, Vol.21, No.8, 1008–1015, 2010, DOI: 10.1016/j.foodqual.2010.05.017.

[36] S. J. Hur, S. Y. Lee, Y. C. Kim, I. Choi, G. B. Kim. Effect of fermentation on the antioxidant activity in plant-based foods, Food Chemistry, Vol.160, No.1, 346-356, 2014, DOI: 10.1016/j.foodchem.2014.03.112.

[37] R. Ojha, Y. Bhurtel, R. Karki, U. Subedi. Processing effects on anti-nutritional factors, phytochemicals, and functional properties of horse gram (Macrotyloma uniflorum) flour, Journal of Microbiology, Biotechnology and Food Sciences, Vol.9, No.6, 1080-1086, 2020, DOI: 10.15414/jmbfs.2020.9.6.1080-1086.

[38] W. Liu, M. Dun, X. Liu, G. Zhang, J. Ling. Effects on total phenolic and flavonoid content, antioxidant properties, and angiotensin I-converting enzyme inhibitory activity of beans by solid-state fermentation with Cordyceps militaris, International Journal of Food Properties, Vol. 25, No. 1, 477 – 491, 2022, DOI: 10.1080/10942912.2022.2048009.

[39] M. Y. Juan, C. C. Chou. Enhancement of antioxidant activity, total phenolic and flavonoid content of black soybeans by solid state fermentation with Bacillus subtilis BCRC 14715, Food Microbiology, Vol.27, No.5, 586-591, 2010, DOI: 10.1016/j.fm.2009.11.002.

[40] B. P. Singh, B. Bhushan, S. Vij. Antioxidative, ACE inhibitory and antibacterial activities of soy milk fermented by indigenous strains of lactobacilli, Legume Science, Vol.2, No.4. 2020, DOI: 10.1002/leg3.54.

[41] S. Aryal, M. K. Baniya, K. Danekhu, P. Kunwar, R. Gurung, N. Koirala. Total phenolic content, flavonoid, content and antioxidant potential of wild vegetables from Western Nepal, Plants (Basel), Vol.8, No.4, 2019, DOI: 10.3390/plants8040096.

[42] C. S. Kwak, M. S. Lee, S. C. Park. Higher antioxidant properties of Chungkookjang, a fermented soybean paste, may be due to increased aglycone and malonylglycoside isoflavone during fermentation, Nutrition Research, Vol.27, No.11, 719-727, 2007, DOI: 10.1016/j.nutres.2007.09.004.

[43] P. K. Sadh, P. Saharan, S. Duhan, J. S. Duhan. Bio-enrichment of phenolics and antioxidant activity of combination of Oryza sativa and Lablab purpureus fermented with GRAS filamentous fungi, Resource-Efficient Technologies, Vol.3, No. 3, 347 – 352, 2017, DOI: 10.1016/j.reffit.2017.02.008.

[44] A. Hashim, M. S. Khan, M. S. Khan, M. H. Baig, S. Ahmad. Antioxidant and α-amylase inhibitory property of Phyllanthus virgatus L.: An in vitro and molecular interaction study, BioMed Research International, 2013, DOI: 10.1155/2013/729393.

[45] G. Oboh, A. O. Ademiluyi, A. A. Akindahunsi. Changes in polyphenols distribution and antioxidant activity during fermentation of some underutilized legumes, Food Science and Technology International, Vol.15, No.1, 41–46, 2009, DOI: 10.1177/1082013208101022.

[46] C. I. Chukwuma, M. G. Matsabisa, M. A. Ibrahim, O. L. Erukainure, M. H. Chabalala, M. S. Islam. Medicinal plants with concomitant anti-diabetic and anti-hypertensive effects as potential sources of dual acting therapies against diabetes and hypertension: A review, Journal of Ethnopharmacology, Vol.235, 329-360, 2019, DOI: 10.1016/j.jep.2019.02.024.

[47] A. E. A. Magro, L. C. Silva, G. B. Rasera, R. J. S. Castro. Solid-state fermentation as an efficient strategy for the biotransformation of lentils: enhancing their antioxidant and antidiabetic potentials, Biosources and Bioprocessing, Vol.6, No.38, 2019, DOI: 10.1186/s40643-019-0273-5.

[48] S. Noviasari, F. Kusnandar, A. Setiyono, S. Budijanto. Antioxidant activity and inhibition of α-amylase and α-glucosidase in fermented black rice bran-based analog rice, AIMS Agriculture and Food, Vol. 7, No. 1, 61 – 72, 2022, DOI: 10.3934/agrfood.2022004.

[49] J. H. Lee, C. E. Hwang, K. S. Son, K. M. Cho. Comparisons of nutritional constituents in soybeans during solid state fermentation times and screening for their glucosidase enzymes and antioxidant properties, Food Chemistry, Vol. 272, 362 – 371, 2019, DOI: 10.1016/j.foodchem.2018.08.052.

[50] X. Lin, Y. Tang, Y. Hu, Y. Lu, Q. Sun, Y. Lv, Q. Zhang, C. Wu, M. Zhu, Q. He, Y. Chi. Y. Sodium reduction in traditional fermented foods: Challenges, strategies, and perspective, Journal of Agricultural and Food Chemistry, Vol.69, 8065-8080, 2021, DOI: 10.1021/acs.jafc.1c01687.

[51] H. Liu, S. Yang, J. Liu, J. Lu, D. Wu. Effect of salt concentration on Chinese soy sauce fermentation and characteristics, Food Bioscience, Vol. 53, 2023, DOI: 10.1016/j.fbio.2023.102825.

[52] C. Niu, X. Xing, F. Zheng, C. Liu, J. Wang, Q. Li. Effect of salt reduction on nutritional, functional and sensory aspects of Anhui-style doubanjiang, a traditional Chinese broad bean paste, Systems Microbiology and Biomanufacturing, Vol.3, 262-272, 2023, DOI: 10.1007/s43393-022-00112-9.

[53] S. S. Kim, J. A. Heo, Y. Kim, M. J. Kim, H. S. Kwak. Salt contents and aging period effects on the physicochemical properties and sensory quality of Korean traditional fermented soybean paste (doenjang), Food Bioscience, Vol. 36, 2020, DOI: 10.1016/j.fbio.2020.100645.

[54] M. Ogasawara, Y. Yamada, M. Egi. Taste enhancer from the long-term ripening of miso (soybean paste), Food Chemistry, Vol.99, No.4, 736-741, 2006, DOI: 10.1016/j.foodchem.2005.08.051.

[55] S. Kremer, J. Mojet, R. Shimojo. Salt reduction in foods using naturally brewed soy sauce, Journal of Food Science, Vol.74, No.6, 255-262, 2009, DOI: 10.1111/j.1750-3841.2009.01232.x.