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Production of Gamma Aminobutyric Acid (GABA)- *Miso* using *Candida parapsilosis* Isolated from a Commercial Soy Sauce *Moromi*

(Penghasilan Asid Gamma Aminobutirik (GABA)- *Miso* menggunakan *Candida parapsilosis* Dipencilkan daripada Kicap Komersial *Moromi*)

Chong Shin Yee[1,2], Zul Ilham[2], Nurul Aqilah Mohd Zaini[3], Muhamad Hafiz Abd Rahim[4], Siva Raseetha [5], António Raposo[6], Sehad N Alarifi[7], Mohd Nizam Lani[8] & Wan Abd AL Qadr Imad Wan-Mohtar[1,*]

[1]*Functional Omics and Bioprocess Development Laboratory, Institute of Biological Sciences, Faculty of Science, University Malaya, 50603 Kuala Lumpur, Malaysia*
[2]*Biomass Energy Laboratory, Faculty of Science, University Malaya, 50603 Kuala Lumpur, Malaysia*
[3]*Department of Food Sciences, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor, Malaysia*
[4]*Faculty of Food and Science and Technology, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*
[5]*Faculty of Applied Sciences, Universiti Teknologi MARA, 40450 Shah Alam, Selangor, Malaysia*
[6]*CBIOS (Research Center for Biosciences and Health Technologies), Universidade Lusófona de Humanidades e Tecnologias, Campo Grande 376, 1749-024 Lisboa, Portugal*
[7]*Department of Food and Nutrition Science, Al-Quwayiyah College of Sciences and Humanities, Shaqra University, Shaqraa 11971, Saudi Arabia*
[8]*Faculty of Fisheries and Food Science, Universiti Malaysia Terengganu, 21030 Kuala Terengganu, Terengganu, Malaysia*

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ABSTRACT

A newly identified yeast, *Candida parapsilosis* strain KBC (CPSKBC) was tested for its GABA-producing potential in *miso* fermentation and consumer acceptability towards the GABA-*miso* was evaluated through sensory analysis. Three different formulations were used over one month to make the GABA-*miso*: formulation 1 (F1) used soybeans, rice *koji*, and *miso* starter; formulation 2 (F2) used soybeans, rice *koji*, and CPSKBC suspension; and formula 3 (F3) used soybeans, rice *koji, miso* starter, and CPSKBC suspension. High Performance Liquid Chromatography (HPLC) was used to measure the gamma-aminobutyric acid (GABA) concentration of the *miso* produced. F3 produced the highest GABA (36.73 mg/L), followed by F2 (34.54 mg/L) and F1 (28.42 mg/L). Sensory evaluation was conducted with 60 panelists using a 9-point hedonic scale. For all the sensory attributes evaluated (appearance, color, odor, taste and overall acceptability), the results showed no significant differences (p >0.05) between groups of *miso* (F1, F2, and F3) and commercial *miso* (C1). We can conclude that our products (F1, F2, and F3) matched with commercially produced miso in the market. This study demonstrated that CPSKBC was able to produce GABA-*miso* (F2 and F3) which is acceptable to the consumer.

Keywords: *Candida parapsilosis*; GABA; *miso*; *moromi*; soy sauce

ABSTRAK

Candida parapsilosis strain KBC (CPSKBC), yis yang baharu dikenal pasti, telah diuji potensinya untuk menghasilkan GABA semasa penapaian *miso* dan penerimaan pengguna terhadap GABA-*miso* telah dinilai melalui analisis deria. Tiga formulasi berbeza telah digunakan dalam tempoh satu bulan untuk membuat GABA-miso: formulasi 1 (F1) menggunakan kacang soya, beras koji dan pemula *miso*; formulasi 2 (F2) menggunakan kacang soya, koji beras dan ampaian CPSKBC; serta formula 3 (F3) menggunakan kacang soya, koji beras, pemula *miso* dan ampaian CPSKBC.

Kromatografi Cecair Prestasi Tinggi (HPLC) digunakan untuk mengukur kepekatan asid gamma-aminobutirik (GABA) *miso* yang dihasilkan. F3 menghasilkan GABA tertinggi (36.73 mg/L), diikuti oleh F2 (34.54 mg/L) dan F1 (28.42 mg/L). Penilaian deria telah dijalankan dengan 60 ahli panel menggunakan skala hedonik 9 mata. Bagi semua atribut deria yang dinilai (penampilan, warna, bau, rasa dan kebolehterimaan keseluruhan), keputusan menunjukkan tiada perbezaan yang ketara ($p > 0.05$) antara kumpulan miso (F1, F2 dan F3) dan miso komersial (C1). Kami membuat kesimpulan bahawa produk kami (F1, F2 dan F3) sepadan dengan miso yang dihasilkan secara komersial di pasaran. Kajian ini menunjukkan bahawa CPSKBC mampu menghasilkan GABA-miso (F2 dan F3) yang boleh diterima oleh pengguna.

Kata kunci: *Candida parapsilosis*; GABA; kicap; miso; *moromi*

Introduction

Miso is a popular traditional Japanese soybean paste that has undergone fermentation. It has a distinctive savoury flavour and aroma and is most used as flavouring in *miso* soup. *Miso* is made by fermenting soybeans with the aid of *koji* which act as a starter mold and salt. According to a study, *miso* was first known as *kokusho* (a paste made up of soybeans and grains which are fermented with salt) in ancient China, and its use later became well-known in Japan during the Edo Period (1603-1868) (Kusumoto et al. 2021). Based on the ingredients used to produce *miso,* it can be categorised into 3 main types: rice *miso*, barley *miso*, and bean *miso*. A study claimed that *miso* is a better and healthier diet that contains peptides, gamma-aminobutyric acid (GABA), fatty acids, fatty acid esters, polysaccharides, glycoproteins, and isoflavones which are created through the breakdown of the ingredients by microorganisms during the fermentation process. Not only that, the metabolic products found in the *miso* claimed to have anti-oxidant, anti-carcinogenic, and anti-hypertensive properties that benefit the consumer (Ohata et al. 2011).

Gamma-aminobutyric acid (GABA) is one of the most researched components found in fermented foods like *miso,* soy sauce, yogurt, and tempeh. Several factors that affect GABA production which includes the raw materials used and also the microorganisms involved in the fermentation process (Herawati et al. 2021). GABA has been shown to have physiological effects on improving both mental health and sleeping quality. However, a study claimed that adding synthetic GABA directly to food, is somewhat risky because it may cause adverse side effects such as addiction, drowsiness, and dizziness in consumers (Hudec et al. 2015). Previous studies have been done to create GABA-rich foods that are safe and natural to be consumed by using different microorganisms in the fermentation process, such as GABA-rich soy sauce (Yee et al. 2021) and GABA-tempeh (Watanabe, Fujimoto & Aoki 2007).

Yeast, molds, and lactic acid bacteria, which make up most of the *miso* microbiota are not only essential to the *miso* fermentation process but also can produce GABA. Like *miso,* several microbes that are found and isolated from the soy sauce *koji* or *moromi* mash are shown to be capable of producing GABA. For example, a fungus known as *Aspergillus oryzae* strain NSK (AOSNSK) is a GABA-producing strain isolated from the KBC soy sauce *koji*, while two lactic acid bacteria namely *Tetragenococcus halophilus* strain KBC (THSKBC) and *Bacillus cereus* strain KBC (BCSKBC) were found to be GABA-producing bacteria which was isolated from the same KBC soy sauce *moromi* mash. To the best of our knowledge, no research had been done yet to look at *Candida parapsilosis'* ability to produce GABA during the fermentation of food like *miso. Candinda parapsilosis* strain KBC is a newly identified yeast strain isolated from the soy sauce *moromi*. There are some studies that showed *Candida parapsilosis* as a yeast commonly found in soybeans fermented foods such as *kinema* (Sarkar et al. 1994), *tungrymbai* (Sohliya et al. 2009), and Japanese *koji* (Allwood, Wakeling & Bean 2021). The research, however, did not go into enough detail about the precise roles and functions of *Candida parapsilosis* in food fermentation. In the present study, a newly identified yeast, *Candida parapsilosis* strain KBC which was isolated from KBC soy sauce *moromi* was studied on its ability to produce and develop GABA-enriched *miso*.

Materials and Methods

Fungal source

Candida parapsilosis was isolated from the 80 days soy sauce *moromi* from Kwong Bee Chun Soy Sauce Sdn.

Bhd., Kamunting, Perak (KBC company), Malaysia using methods described by Khairil Anwar, Idris and Hassan (2020) and Wan-Mohtar, et al. (2020) with slight modification.

ISOLATION OF *Candida parapsilosis* STRAIN KBC

For the isolation of *C. parapsilosis*, 1 mL of 80 days soy sauce *moromi* from KBC company was diluted with 0.85 % saline (9 mL) and a vortex was used to homogenise the solution. 100 µL of the diluted sample was placed on Malt Extract Agar that had been supplied with 5% NaCl (food grade) and 50 mg/mL chloramphenicol (220551-25G, Sigma-Aldrich, Dorset, UK) and incubated at 30 °C for 3-5 days until the single colony is being observed. This procedure was done in an aseptic and sterile environment which was inside a laminar flow chamber (Khairil Anwar, Idris & Hassan 2020).

MOLECULAR IDENTIFICATION

For yeast species identification, *Candida parapsilosis* was identified by using fungal DNA Barcoding by Apical Scientific Sdn. Bhd. (Sri Kembangan, Selangor, Malaysia) employing the method adapted by Ahmad et al. (2021).

MORPHOLOGICAL IDENTIFICATION

Gram staining was used to determine the cell shape and organization of the yeast. A single colony of the yeast from the Malt Extract Agar plate was transferred to a slide and heat was applied to heat-fix the smear. After heat-fixing the smear, a few drops of crystal violet staining reagent were used to stain the smear for 60 s. The slide was then rinsed with distilled water and iodine was applied which acts as a mordant and the dye were for another 60 s. A few drops of 95 % of alcohol were used to rinse and decolorizing the smear for around 10-15 s, followed by a few drops of safranin to cover the smear for another 60 s. The slide was carefully rinsed with distilled water to remove excess safranin and dried off using absorbent paper. The slide was observed under 400x magnification by using a light microscope (Leica DM 750, Leica Microsystem, Wetclar, Germany) with immersion oil.

SUSPENSION OF *Candida parapsilosis* STRAIN KBC

Three loopfuls of *Candida parapsilosis* strain KBC were mixed with 20 mL of distilled water in a 50 mL centrifuge tube. The mixture was then vortex for 3 min to get a homogenized *C. parapsilosis* strain KBC inoculum suspension. In order to get the approximate number of yeast cells from the inoculum suspension, the turbidity of the yeast suspension was compared with the 0.5 McFarland standard, which was equivalent to approximately 1.3×10^6 CFU/mL of yeast suspension. 0.5 McFarland standard will have an absorbance (OD_{600}) of about 0.12 - 0.15 when measured by using a spectrophotometer. By serial dilution or additional yeast inoculation, the turbidity of the yeast suspension was adjusted until it fitted the standard (Guinea et al. 2010).

MISO MAKING

After soaking 125 g of soybeans overnight, the soybeans were cooked for 4-5 h at 100 °C until they turned soft. Next, the soybeans were taken out from the pot and cool to room temperature which was between (35 °C - 40 °C) and the cooled soybeans were blended into a paste by using a food processor. To make the *miso*, 100 g of the rice *koji* was soaked in 50 mL water with 50 g additional salt for 20 min. The soaked rice *koji* was combined with additional components and added to the bean paste following three different formulas: Formula 1 (F1) was inoculated with 10 g of *miso* starter; Formula 2 (F2) was inoculated with 10 mL of *C. parapsilosis* strain KBC suspension; Formula 3 (F3) was inoculated with 10 g of *miso* starter and 10 mL of *C. parapsilosis* strain KBC suspension. The mixture was mixed properly for 10 min to get a soft appearance and dough texture. To remove all the air in the *miso* jar, the mixture was squeezed and formed into a ball shape and all the ball mixtures were then placed and pressed down tightly in the 1 L air-tight jar. The surface of the *miso* paste was covered with salt before being wrapped in a wrapping film to keep out the air. Before sealing the container's lid, a weight was placed on the top of the wrapping film. The *miso* was fermented for 31 days at 30°C according to Saio et al. (1984) method with slight modification. For each formulation, the experiment was conducted independently in triplicate.

GABA IDENTIFICATION

High-performance liquid chromatogram (HPLC) had been carried out to analyse the GABA concentration in each sample according to Xu et al. (2017) method with slight modification.

SENSORY ANALYSIS OF THE MISO

The sensory analysis was carried out with 60 panelists (students and staff) from the Faculty of Science, University

of Malaya, Kuala Lumpur, Malaysia according to Wan-Mohtar (2021) method with some modifications. Prior to taking part in the study, panelists were asked about their health such as allergies. The panelist was given *miso* soup made from the 3 different formulations of *miso* from this study (F1, F2, and F3) and the commercial *miso* (C1). The samples of *miso* soup were made by dissolving 20 g of each *miso* paste (F1, F2, F3, and C1) in 400 mL of water, then boiling the soup for 10 min, according to Inoue et al. (2016) with a minor modification. About 5 mL of each sample of *miso* soup which was presented in three-digit blinding codes was served simultaneously with a cup of water for mouth rinsing among samples to each panelist in random order. The sample of *miso* soup was evaluated using a 9-point Hedonic scale (1 = extremely poor, 2 = very poor, 3 = poor, 4 = below fair above poor, 5 = fair, 6 = below good above fair, 7 = good, 8 = very good, 9 = excellent).

The panelists were asked to fill out the Google Form and submitted it at end of the analysis. 60 panelists evaluated only the *miso* soup samples, scoring them on a 9-point hedonic scale for appearance, color, odor, taste, and overall acceptability. The *miso* paste was provided only for observation purposes.

STATISTICAL ANALYSIS

Software from IBM SPSS (version 28) was utilized to analyse the statistical data from this study. One-way ANOVA with Post Hoc tests was used to detect the significant difference between mean value using Tukey's test with a 95% confidence level. Results with a *p-value* of 0.05 or higher were considered significantly different.

Results and Discussion

MOLECULAR IDENTIFICATION OF *Candida parapsilosis* STRAIN KBC (CPSKBC) ELECTROPHORESIS *of* CPSKBC

C. parapsilosis was identified by using fungal DNA Barcoding by Apical Scientific Sdn. Bhd (Sri Kembangan, Selangor, Malaysia) according to the method from Ahmad et al. (2021) with few modifications. Lane 1 and Lane 5 were designed as standard curves (M) for determining the length of base pairs as shown in Figure 1, and then matched with the 10 kb marker. Lane 2 showed the negative control (-ve), while the positive control (+ve) is shown in Lane 3. Lane 4 showed the sample of the species *C. parapsilosis* (CP) which has a similar range of bp size of 500bp and 750bp to the positive control (Lane 3). Thus, the base pair for the CP was calculated to be 529 bp (Figure 1).

To create a phylogenetic tree, top-10 related species were extracted from the NCBI Gene Bank and blasted with the isolation of (*C. parapsilosis* strain KBC). According to the reference database, which is shown in Figure 2, *C. parapsolosis* strain KBC was classified in the same clade (Clade A) as *C. parapsilosis* SNPLPY2, and this showed that this species is phylogenetically close to *C. parapsilosis*. The *C. parapsilosis* strain KBC (529 bp) was verified by using plasmid matching (ApE) software which the sequence of the species is compared with *C. parapsilosis* strain CBS 604 18S (2782 bp). There was no mismatched and unknown base pair found between the two species, hence it was shown that the *C. parapsilosis* strain KBC has 98% similarities with the *C. parapsilosis* strain CBS 604 18S.

Morphological Characteristic of C. parapsilosis KBC

Morphological characteristics of the yeast strain isolated from the 80 days soy sauce *moromi* are illustrated in Figure 3. On the Malt Extract Agar plates, the isolated strain produced colonies that have mixed morphotypes which were smooth-glossy, and crepe as indicated in Figure 3(A). The smooth colonies are primarily made up of yeast-form cells as shown in Figure 3(B), while non-smooth or crepe colonies are primarily made up of pseudo hyphal cells or a combination of both morphologies as shown in Figure 3(C). The Gram staining further demonstrated that this strain for both yeast and pseudo hyphae is Gram-positive. The pseudo hyphae are extended yeasts cells that have the appearance of hyphae but are not the actual hyphae.

MISO FERMENTATION

Miso produced from Formulation 1 (F1: Soybean + Rice *koji* + *miso* starter) before and after one month are shown in Appendix 1 (F1a and F1b, respectively); *Miso* produced from Formulation 2 (F2: Soybean + Rice *koji* + CPSKBC suspension) before and after one month are shown in Appendix 1 (F2a and F2b, respectively); *Miso* produced from Formulation 3 (F3: Soybean +Rice *koji* + *miso* starter CPSKBC suspension) before and after one month are shown in Appendix 1 (F3a and F3b, respectively).

GABA ANALYSIS

The GABA content in the *miso* was measured through HPLC after one month of *miso* fermentation. According to Table 1, Formulation 3 (Soybean+ Rice *koji* + *miso* starter CPSKBC suspension) produced the highest GABA concentration which was 36.73 mg/L, followed

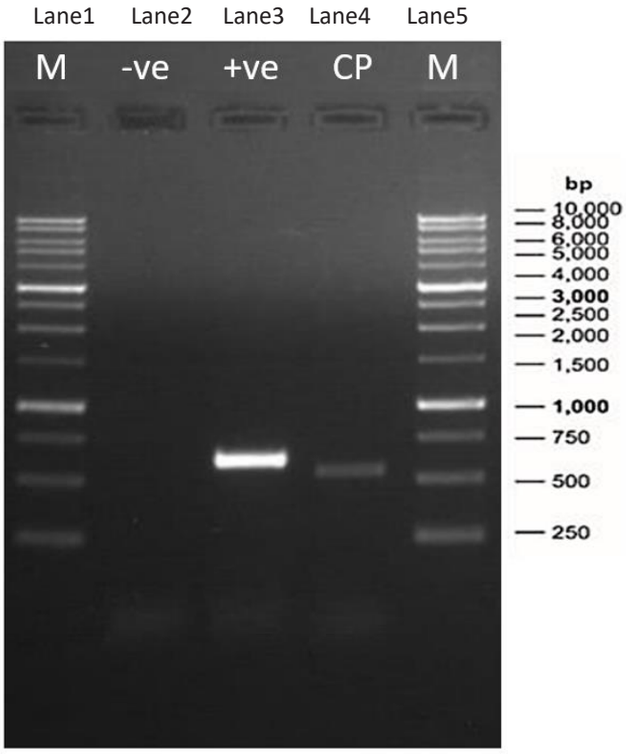

FIGURE 1. *C. parapsilosis* (CPSKBC) culture running through agarose gel electrophoresis of 16S rRNA isolated from. Lane 1 and lane 5 indicate to 10kb marker. Positive control (+ve) was indicated in Lane 3 while the negative control (-ve) was indicated in Lane 2. Lane 4 indicates the sample which is the *C. parapsolosis* (CP)

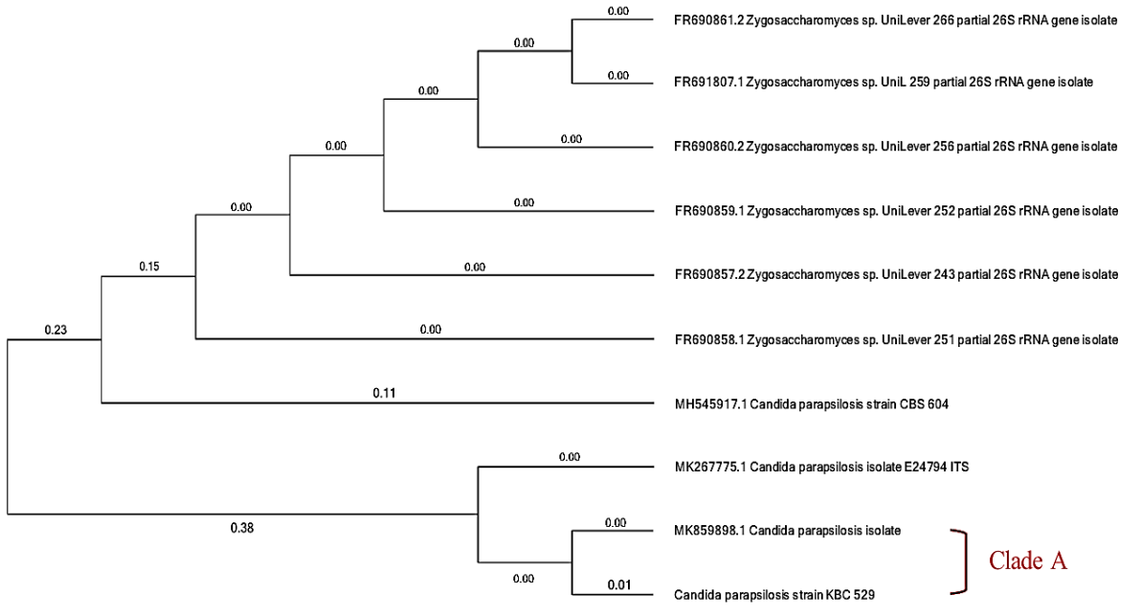

FIGURE 2. Phylogenetic tree of *C. parapsilosis* strain KBC. *C. parapsolosis* strain KBC was classified in the same clade (Clade A) as *C. parapsilosis* SNPLPY2, and this shows that this species is phylogenetically close to *C. parapsilosis*

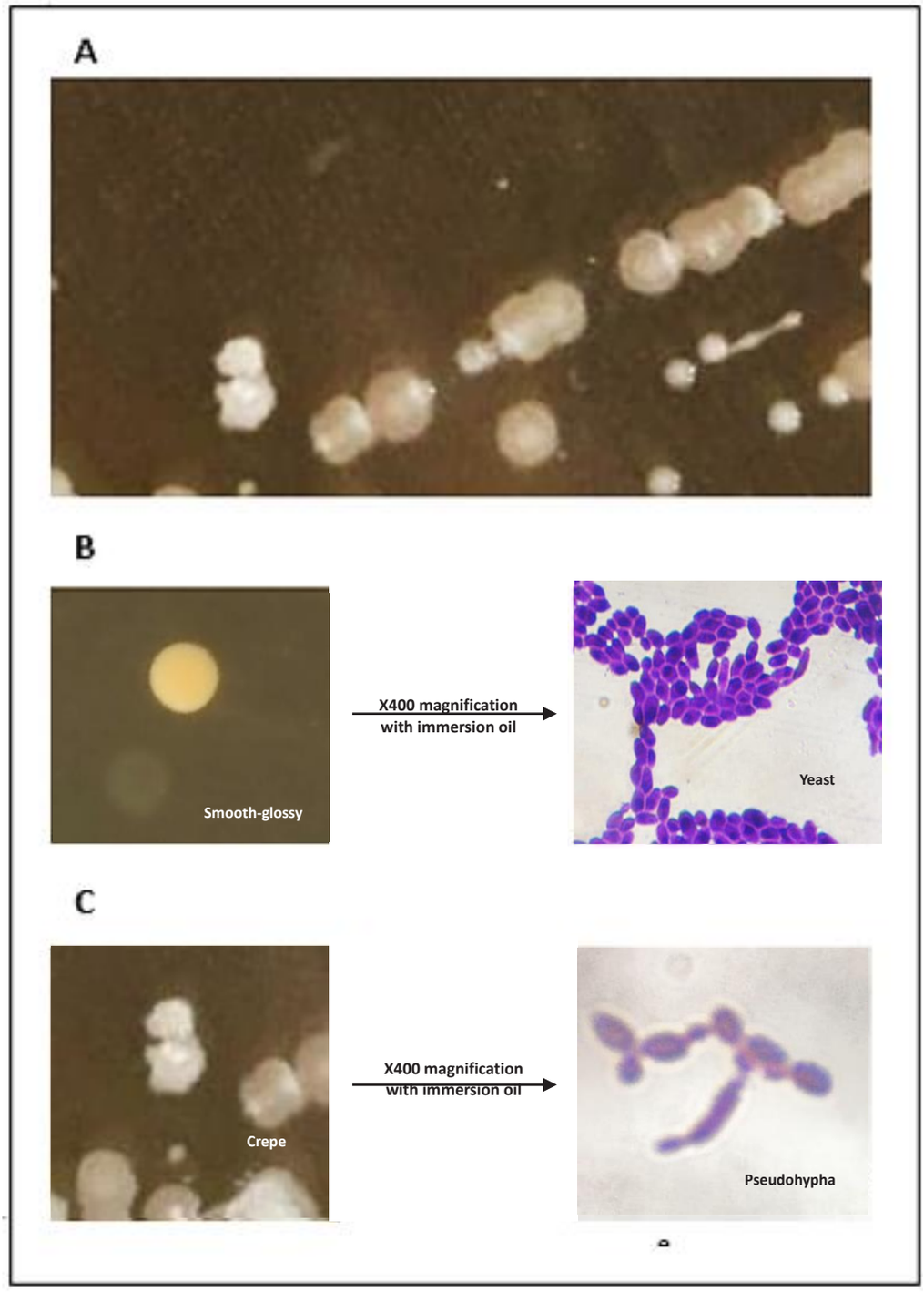

FIGURE 3. Colonies of isolated yeast strain from the soy sauce moromi (A) Mixed morphology observes on the Malt Extract Agar plate. (B) Smooth colonies are primarily made up of yeast-form cells. (C) Non-smooth colonies are primarily made up of pseudohyphal cells

by Formulation 2 (Soybean+ Rice *koji* + CPSKBC suspension) which was 34.54 mg/L and Formulation 1 (Soybean+ Rice *koji* + *miso* starter) which was 28.42 mg/L. To the best of our knowledge, no study was done to investigate the capability of *C. parapsilosis* in producing GABA. In this study, *miso* inoculated with CPSKBC (F2 and F3) produced higher GABA compared to the *miso* that was not added with CPSKBC (F1). This study suggests that the CPSKBC which was isolated from the soy sauce *moromi* was a potential GABA producer.

The species and strain of microorganisms differ in their capacity to produce GABA, thus more research on the identification and isolation of GABA-producing microorganisms is required as these microbes are beneficial to the food industry. Lactic acid bacteria (LAB) were found to be more common in fermented foods and to be used in the production of fermented foods due to their food-grade nature and capacity to produce GABA (Cui et al. 2020). Selecting the right LAB isolate for GABA production can result in beneficial health benefits. Previous research demonstrated that a daily dose of 10 mg of GABA helped hypertensive individuals lower their blood, while a daily dose of 26 mg of GABA reduced depression and enhanced sleep (Carafa et al. 2019, Okada et al. 2000). *Bacillus cereus* strain KBC and *T. halophilus* strain KBC which were isolated from the same soy sauce *moromi* mash also found to be significant GABA producers in soy sauce fermentation. Previous study showed that both of these LAB were able to produce high GABA (161 and 159 mg/L, respectively) in the soy sauce fermentation (Yee et al. 2021). Furthermore, several research have been carried out to examine the GABA-producing capacity of LAB isolated from other sources such as *kung-som* (Thai fermented shrimp condiment), Funa-sushi (fermented fish), kimchi, cheese and cheese starter, and GABA concentration were significantly raised in the most cases as a result of LAB inoculation in suitable culture medium (Di Cagno et al. 2010, Komatsuzaki et al. 2005, Lee et al. 2010; Sanchart et al. 2018). According to the Food and Drug Administration (FDA), a selected lactic acid bacteria strain, with a 'generally recognized as safe' (GRAS) status, shown significant impact under high salt concentrations makings it useful for producing GABA-enrich functional food or soy products (Sassi et al. 2022).

Aspergillus oryzae, a well-known filamentous mold that has been used to produce soy sauce was found to produce a significant amount of GABA under unoptimized as well as optimized conditions ranging from 73.13 to 3278.31 mg/L (Ab Kadir et al. 2016; Wan-Mohtar et al. 2019). Additionally, according to Cai et al. (2014), the GABA concentration of the fermented oats like *tempeh* produced through fungal fermentation by the *Aspergillus oryzae* 3.5232 after 72 h was about 8 times greater (435.8 µg/g oats) when compare to the native oats which is only 57.1 µg/g oats. These researchers have shown that the GABA-producing fungus *A. oryzae* is a GABA-producing mold.

Studies also proved that not only mold but yeast have the capability in producing GABA. Li et al. (2022) found GABA-producing yeasts such as *Kluyveromyces marxianus* B13-5 and *Saccharomyces cerevisiae* DL6-20 which was isolated from the Kazakh cheese have the ability to increase GABA production during the cheese fermentation. Besides, Zhang et al. (2022) found another GABA-producing yeast known as *K. marxianus* C21 was able to produce the highest GABA of 4310 mg/L under optimized conditions in okara fermentation. Generally, the GABA-producing microbes synthesis the glutamate decarboxylase (Ahmad et al. 2021) to enable the conversion of glutamic acid into GABA via catalysis (Cai et al. 2014; Wan-Mohtar et al. 2019). In this present study, the yeast CPSKBC has been proven to exhibit similar effects in terms of GABA production. This may be due to the presence of glutamic acid decarboxylase (Ahmad et al. 2021) activity in CPSKBC.

TABLE 1. The GABA concentration after one-month fermentation

Sample	GABA concentration (mg/L)
F1: Soybean + Rice *koji* + *miso* starter	$27.47^{a} \pm 0.92$
F2: Soybean + Rice *koji* + CPSKBC suspension	$34.12^{a} \pm 0.43$
F3: Soybean +Rice *koji* + *miso* starter CPSKBC suspension	$*36.80^{a} \pm 0.28$

Means with the same superscript alphabets within the identical column indicate a significant difference ($p < 0.05$). The symbol asterisk (*) shows the highest mean value

SENSORY ANALYSIS

Five sensory attributes for *miso* had been chosen for this study. This included 'appearance', 'colour', 'odour', 'taste' and 'overall acceptability'. Figure 4 shows a spider web of the average ratings for the five sensory attributes assessed from all samples of *miso* soup made by using each formulation. According to Table 2, the formulations were assigned as F1, F2, F3, and C1 in the sensory analysis. The ANOVA shows no significant difference between the groups of *miso* made from 3 different formulations and commercial *miso* in terms of appearance, colour, odor, taste, and overall acceptance ($p > 0.05$). Based on the findings, it can be concluded that the 3 *miso* soup samples were accepted by the panelist almost equally as well as commercial *miso*.

Miso soup made with F1 had the highest mean scores for appearance, colour, and odor which is 6.17, 6.23, and 6.05, respectively, when compared to other *miso* samples. However, F2 *miso* soup received a higher mean score for the taste which was 5.57 when compared to other *miso* samples. F1, F2, and F3 show no difference in overall acceptability, however, the commercial *miso* had lower ratings for odour, flavour, and overall acceptability which is 5.53, 4.60, and 4.98, respectively, when compared to the other *miso* samples (F1, F2, and F3). This may due to the stronger flavor and smell, which some panelist may find less appetizing or bearable. Commercial *miso* usually ferments for 2 to 24 months which is much longer than that of the *miso* paste produced in this study. Thus, longer fermentation time results in a deeper colour, richer and stronger flavour formation (Allwood, Wakeling & Bean 2021). The overall findings showed that *miso* soup created from F1, F2, and F3 were comparable with commercially produced *miso* in the Malaysian market at no significant difference ($p < 0.05$). *Miso* soups produced with F1, F2, and F3 score very similar for odor and taste, which the difference between any two being less than 0.5. From the slight difference in the score of odor and taste between the F1, F2, and F3, we can conclude that the inoculation of CPSKBC in *miso* making did not result in a significant impact on the sensory characteristics, especially for the odor and taste enrichment. Yet, it does not affect the odor and taste of the *miso* negatively. The result from this study was not correlated with the study by Jiang et al. (2021) which indicates that *C. parapsilosis* was one aroma-producing yeasts isolated from soy sauce *moromi* that considerably enhanced the flavor, aroma, and taste of soy sauce. But this research showed that *C. parapsilosis* strain KBC does not enhance the flavour and taste in the fermentation of *miso*. More studies should be done in the future to investigate the precise roles and responsibilities of *C. parapsilosis* in the fermentation of *miso*.

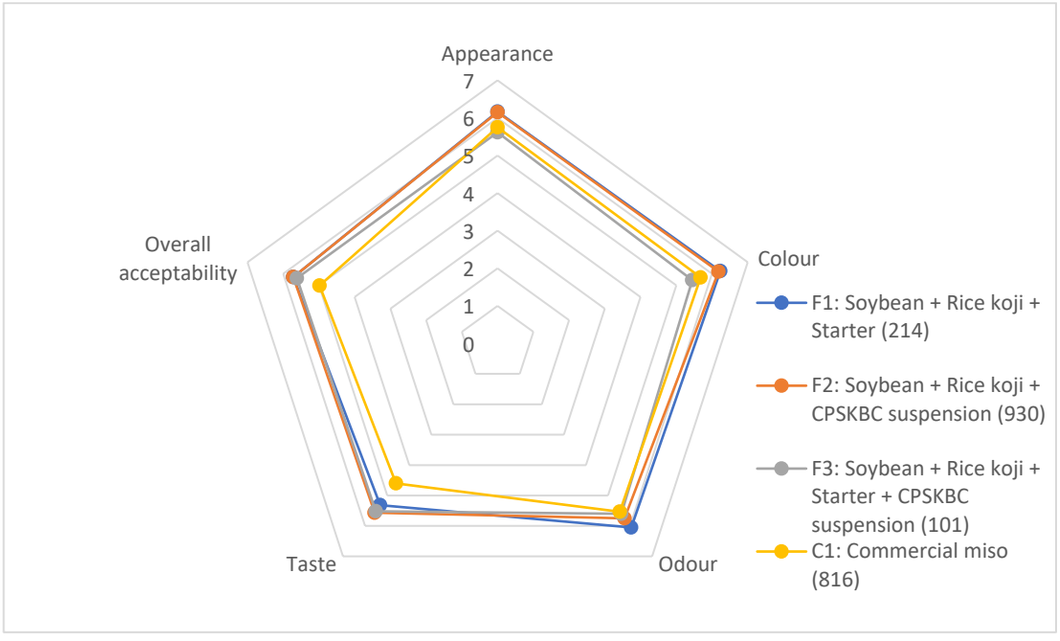

FIGURE 4. Respondents' preference of three different samples of miso soup using spider web (n = 60)

TABLE 2. Hedonic nine-point scale preference scores for each attribute of *miso* soup samples (1 = extremely poor, 2 = very poor, 3 = poor, 4 = below fair above poor, 5 = fair, 6 = below good above fair, 7 = good, 8 = very good, 9 = excellent)

Formulation	Preference scores (Mean ± SD)				
	Appearance	Color	Odor	Taste	Overall acceptability
F1: Soybean + Rice *koji* + *Miso* starter (214)	*6.17 ± 2.00[a]	*6.23 ± 2.21[a]	*6.05 ±2.16[a]	5.32 ± 2.49[a]	*5.72 ± 2.23[a]
F2: Soybean + Rice *koj*i + Yeast (930)	6.15 ± 2.14[a]	6.18 ± 2.20[a]	5.75 ± 2.25[a]	*5.57 ± 2.36[a]	*5.72 ± 2.24[a]
F3: Soybean + Rice *koji* + *Miso* Starter + Yeast (101)	5.62 ± 1.97[a]	5.45 ± 2.17[a]	5.60 ± 2.00[a]	5.52 ± 2.30[a]	5.62 ± 2.12[a]
C1: Commercial *miso* (816)	5.75 ± 2.30[a]	5.68 ± 2.49[a]	5.53 ± 2.33[a]	4.60 ± 2.61[a]	4.98 ± 2.38[a]

Means with different superscript alphabets within the identical column indicate significant difference ($p < 0.05$). The symbol asterisk (*) shows the highest mean value

Conclusions

GABA-enriched *miso* can be produced using *C. parapsilosis* strain KBC (CPSKBC) isolated from a commercial soy sauce *moromi*. Based on the sensory evaluation, we can conclude that our products (F1, F2, and F3) were comparable with commercially produced *miso* in the Malaysian market. There is no significant difference seen in the appearance, colour, odor, taste, and overall acceptance between the groups of *miso* produced using 3 different formulations and commercially produced ($p > 0.05$). Based on the results obtained, it can be inferred that the 3 *miso* soup samples were received with a comparable level of acceptance by the panelists, similar to the commercial *miso*. Therefore, this study not only suggested that yeast was able to ferment *miso* but also able to produce GABA during the *miso* fermentation. However, there is a need for further research that will take advantage of the full potentials offered by the newly identified yeast in *miso* fermentation.

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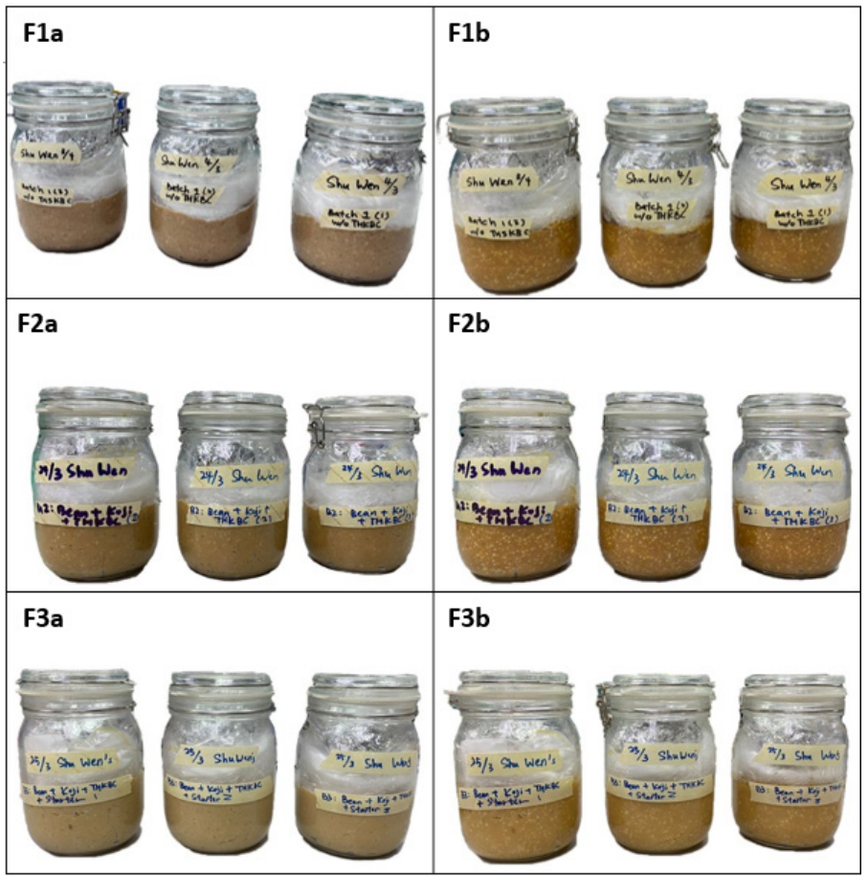
*Corresponding author; email: qadyr@um.edu.my



APPENDIX 1. *Miso* produced from Formulation 1 before (F1a) and after one month (F1b); *Miso* produced from Formulation 2 before (F2a) and after one month (F2b); *Miso* produced from Formulation 3 before (F3a) and after one month (F3b)