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Effect of Aging Duration on Antidepressant Activity and Sensory Properties of Whey Kefir-Fermented Solo-Bulb Black Garlic

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Abstract. Depression is a prevalent mental disorder worldwide, and conventional antidepressants often cause side effects that reduce patients' quality of life. Therefore, developing safer natural alternatives is essential. Solo-Bulb Black Garlic (SBBG), a fermented single-clove garlic, exhibits various health benefits, including potential antidepressant activity. This study evaluated the antidepressant effect of whey kefir–fermented SBBG aged for 7, 14, and 21 days in male mice subjected to chronic mild stress (CMS). Antidepressant activity was assessed using the Forced Swimming Test (FST) by measuring immobility, swimming, and climbing behavior, while leukocyte and erythrocyte counts indicated immune responses. The SBBG-21 group exhibited the greatest reduction in immobility duration (1.08 ± 0.30 minutes), accompanied by increased swimming (1.39 ± 0.11 minutes) and climbing (1.53 ± 0.20 minutes) durations. Leukocyte (7.89×10^3 cells/µL) and erythrocyte (6.19×10^6 cells/µL) counts were close to normal levels. Organoleptic scores for SBBG21 were highest for color (3.54±1.29), aroma (3.17±1.29), taste (3.00 ±1.28), texture (3.35±1.08), and overall preference (3.20±1.11). These results indicate that whey kefir-fermented SBBG aged for 21 days has potential as a functional food with antidepressant properties. It also offers a safer natural alternative to conventional antidepressants, with good acceptability and the potential to improve mental health and quality of life. Furthermore, this research supports SDG_s 3 by promoting alternative strategies for managing depression and SDG_s 12 by utilizing natural fermentation processes and value-added agricultural products.

Keywords: aging; antidepressant; organoleptic; solo-bulb black garlic; whey kefir

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INTRODUCTION

Depression is a common mental disorder and a major global public health concern. In Indonesia, depression affects many individuals, and its prevalence is expected to increase as the population grows (Mustamin et al., 2022). Depression causes persistent sadness, loss of interest in daily activities, and disturbances in sleep, appetite, and energy levels. These symptoms can severely reduce the quality of life. Although depression has long been associated with dysregulation of serotonin and norepinephrine, new findings show that these neurotransmitters do not fully explain its complexity. Recent studies emphasize that glutamate and gamma-aminobutyric acid (GABA) regulate synaptic plasticity, neuronal adaptation, and emotional processing, contributing to depression's development and progression (Sarawagi et al., 2021).

Although antidepressant medications remain the primary treatment for depression, their use is often associated with adverse effects that may reduce patient adherence and quality of life. Antidepressants, including tricyclic antidepressants, selective serotonin reuptake inhibitors (SSRIs), and monoamine oxidase inhibitors (MAOIs), exert their therapeutic effects by modulating neurotransmitter levels, particularly serotonin and norepinephrine (Bashir et al., 2022). These drugs are widely prescribed for depression and several other clinical conditions due to their broad pharmacological effects (Skånland & Pobuda, 2019). However, their use is frequently associated with adverse effects, including urinary retention, constipation, blurred vision, tachycardia, dry mouth, orthostatic

hypotension, nausea, and vomiting, which may negatively affect patients' quality of life (Dipiro et al., 2015). Consequently, there is growing interest in exploring natural products with antidepressant potential. One promising candidate is solo black garlic, a fermented product derived from single-clove garlic that exhibits stronger antioxidant activity than fresh garlic. Its rich content of bioactive compounds may help reduce oxidative stress, a key factor in the pathogenesis of depression, thereby supporting its potential as a natural antidepressant agent.

Bioactive compounds in black garlic, including S-allyl cysteine (SAC), polyphenols, alkaloids, and flavonoids, act as antioxidants that reduce tissue damage and inflammation (Ha & Kim, 2017). SAC and polyphenols inhibit proteolytic enzymes and lower prostaglandin E2 levels, a principal inflammatory mediator. Single-bulb black garlic (SBBG) has been shown to significantly reduce pro-inflammatory cytokines (IL-1β, IL-6, TNF-α) and increase IFN-γ, suggesting immunomodulatory and antidepressant potential through the modulation of inflammation associated with depression (Saryono et al., 2023). Additionally, SBBG contains higher concentrations of allicin and phenolic compounds, further enhancing its anti-inflammatory properties.

A novel fermentation technique involves soaking single garlic cloves in whey kefir for 7 days before aging, thereby increasing the levels of probiotics and bioactive compounds, including SAC and polyphenols. This method extends the findings of Afiati et al. (2020), who reported that soaking garlic in kombucha, black tea, and green tea enhanced both bioactive compound levels and antioxidant activity. In the present study, whey kefir soaking was employed to enhance the bioactivity of solo garlic before black garlic aging. Both black garlic and whey kefir possess well-established antioxidant and health-promoting properties, particularly in mitigating oxidative stress associated with depression. This study assessed the antidepressant effects of whey kefir-fermented solo black garlic in male mice subjected to chronic mild stress, with the objective of advancing functional food strategies for improving mental health.

METHODS

Preparation of Object Animals

The test animals included 24 male Swiss Webster mice (*Mus musculus*) weighing 20-35 grams and aged 12-14 weeks, obtained from the Pharmacotherapy and Therapeutics Laboratory of Padjadjaran University (UNPAD), Bandung, Indonesia (Approval No. 774/UN6.KEP/EC/2025, Registration No. 2507020818). The mice were placed in six treatment cages and acclimatized for 7 days. All mice were healthy, with no abnormalities in their fur, coat color, eyes, or physical condition. During adaptation, the mice were provided special mouse food and water *ad libitum* (OECD, 2025).

Fermenting Single Garlic with Kefir Whey

The fermentation process utilizing whey kefir in this study was adapted from the method of Afiati et al. (2020), with modifications to employ whey kefir as the soaking medium. The soaking procedure for single garlic followed protocols established in previous research. Approximately 2.5 kg of single garlic, imported from China and obtained from traders in Semarang, Central Java, was used. Uniform single garlic bulbs, with diameters ranging from 23 mm to 26 mm, were selected and stored in a dry environment to prevent deterioration before processing. Each cleaned sample, weighing 800 g, was immersed in whey kefir at a 1:1 ratio for seven days. The whey kefir was obtained from the Halal Teaching Factory Laboratory, UIN Sunan Gunung Djati Bandung.

Solo-Bulb Black Garlic Aging Process

The aging process was adapted from the method described by Solichah & Herdyastuti (2021), with modifications. After whey kefir fermentation, the single garlic cloves were drained and weighed to ±200 grams. Next, 300 mL jars were placed on tissue paper to absorb moisture generated during heating. Single garlic cloves were weighed and placed into a glass jar as an experimental unit. The jars were then tightly sealed with aluminum foil to maintain humidity. Finally, the single garlic cloves were fermented in an oven at 60-70°C and 75-85% humidity for 7, 14, or 21 days, depending on the treatment.

Solo-Bulb Black Garlic Extraction

Extraction was performed using the maceration method. The dried solo black garlic sample was placed in a maceration container, and 96% ethanol was added until all samples were submerged (solvent ratio 1:4 w/v). This mixture was stored for 5 x 24 hours with occasional stirring, then the residue was separated from the filtrate. The filtrate was then concentrated using a

Rotary Evaporator at 40 °C and 100 rpm to form a thick extract, which was stored in a closed container. According to Medik et al. (2026), the yield of the thick extract was calculated using the following equation:

$$\%Yield = \frac{\text{Weight of thick extract (g)}}{\text{Weight of initial sample (g)}} \text{ x } 100$$

Research Treatment Phase

Following a 7-day acclimatization period, the male mice were randomly assigned to six treatment groups. The normal control group (C+) received distilled water (*aquadest*) without stress induction, whereas the negative control group (C−) received distilled water following stress induction. The SBBG-7, SBBG-14, and SBBG-21 groups received solo-bulb black garlic extract fermented with whey kefir for 7, 14, and 21 days, respectively, at a dose of 400 mg/kg body weight. Body weight was measured, and mice were subjected to various Chronic Mild Stress (CMS) stressors for 14 days (Qalbiy et al., 2020). From day 15 to day 28, the test preparations were administered orally once daily at the same time using a probe.

Antidepressant activity was then evaluated using the Forced Swimming Test (FST) on days 0, 14, and 28. Leukocyte and erythrocyte levels were also assessed by taking blood samples on day 28. This treatment was accompanied by measuring the body weight of the mice every 3 days.

Antidepressant Activity Testing

Antidepressant activity was assessed using the Forced Swimming Test (FST) on days 0, 14, and 28, following an acclimatization period. Testing was performed before chronic mild stress (CMS) induction and one day after the completion of CMS induction and administration of the test preparation. Three parameters were measured: immobility duration, swimming activity, and climbing (Figure 1). Each mouse was placed in water for 6 minutes, and the durations of immobility, swimming, and climbing were recorded from the 2nd to the 6th minute. Immobility was defined as the period during which the mouse remained motionless with its head above the water surface. During the FST, swimming was defined as active horizontal movement in the water, climbing as vigorous upward movement against the container walls, and immobility as floating with minimal movement to keep the head above the water surface. Immobility was considered an indicator of depression-like behavior (Petković & Chaudhury, 2022).

Table 1. Schedule of Chronic Mild Stress (CMS) Induction

Days	Stress Exposure
Monday	45° tilted cage for 3 hours + 80% reduction in rice husks for 24 hours
Tuesday	20-minute shock + 50% wet rice husks for 24 hours
Wednesday	18-hour restricted food access + 80% reduction in rice husks for 24 hours
Thursday	36-hour continuous light + 45° tilted cage for 3 hours
Friday	1-hour empty bottle exposure + 50% reduction in water and food for 24 hours
Saturday	50% wet rice husks for 21 hours + restricted food access
Sunday	20-minute shock + 80% reduction in rice husks for 24 hours

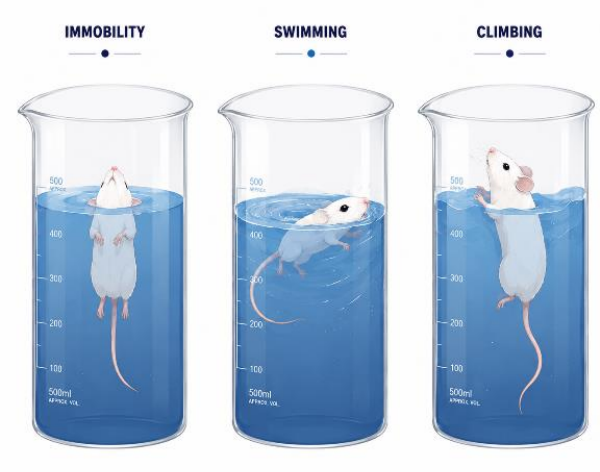

Figure 1. The Forced Swim Test (FST) as a Model of Depressive-like Behavior

Hematological Analysis

On the final day of the experiment, blood samples were collected from the tail veins of mice into EDTA-treated tubes. Leukocyte and erythrocyte counts were measured using a double-improved Neubauer hemocytometer. For leukocyte analysis, blood was drawn to the 0.5 mark of an erythrocyte pipette, mixed with Turk's solution to the 11 mark, and homogenized using a figure-eight motion. The mixture was then loaded onto the hemocytometer, and leukocytes in the four large corner squares were systematically counted under a microscope. The total leukocyte count was calculated using the following equation:

$$N = \mathrm{n} \times 50$$

where *n* is the number of leukocytes in 64 small squares, and *N* is the number of leukocytes per mm³ of blood. For erythrocytes analysis, blood was drawn to 0.5, mixed with Hayem's solution to 101, homogenized using the same procedure, and counted in five large fields (each containing 16 small squares). Total erythrocyte count was calculated using the following equation:

$$N = n \times 10^4$$

where *n* is the number of erythrocytes counted in 80 small squares, and *N* is the number of erythrocytes per mm³ of blood (Maftuch, 2018). Erythrocyte counts are expressed as $\times 10^6$ cells/µL.

Organoleptic Evaluation

The organoleptic properties of whey kefir–fermented SBBG were assessed by 46 semi-trained panelists using a 5-point hedonic scale to evaluate color, aroma, taste, texture, and overall preference (Yuan et al., 2022). The panelists' assessments provided insight into changes in sensory quality over different aging periods.

Data Analysis

Data were analyzed quantitatively using one-way Analysis of Variance (ANOVA), followed by Duncan's multiple range post hoc test. Statistical significance was set at $p < 0.05$. All statistical analyses were performed using SPSS version 26.0 (IBM Corp., Armonk, USA).

RESULTS AND DISCUSSION

Effect of Aging Time of Whey Kefir–Fermented Solo-Bulb Black Garlic

The Forced Swimming Test (FST), a validated method for evaluating depressive-like behavior and antidepressant efficacy in rodents (Armario, 2021), was employed in this study. Mice were placed in water, and their immobility, swimming, and climbing behaviors were recorded. The FST was administered on days 0, 14, and 28. Body weight was monitored throughout the experiment to evaluate the physiological effects of stress. On day 28, leukocyte and erythrocyte counts were determined to assess alterations in blood parameters resulting from stress exposure.

Immobility Duration in Chronic Mild Stress–Induced Mice Treated with Solo-Bulb Black Garlic Extract

The effects of aged black garlic fermented with whey kefir on depressive-like behaviors in male mice were evaluated, with immobility time in the swimming test serving as the primary indicator. Induction of chronic mild stress (CMS) significantly increased immobility duration in negative control groups, confirming the presence of depression-like behavior. Conversely, treatment with amitriptyline and all solo-bulb black garlic extract formulations, including the longest-aged variant (SBBG21), substantially reduced immobility duration, demonstrating pronounced antidepressant-like effects and behavioral recovery. Aged and fermented solo black garlic is rich in phenolic compounds and S-allyl cysteine, both of which exhibit potent antioxidant and anti-inflammatory properties (Ülger & Çakıroğlu, 2024). These bioactive compounds contribute to reducing oxidative stress, a key factor in depression models. The present findings align with previous evidence indicating that antioxidant-rich functional foods can modulate stress responses and promote brain health (Huang et al., 2019).

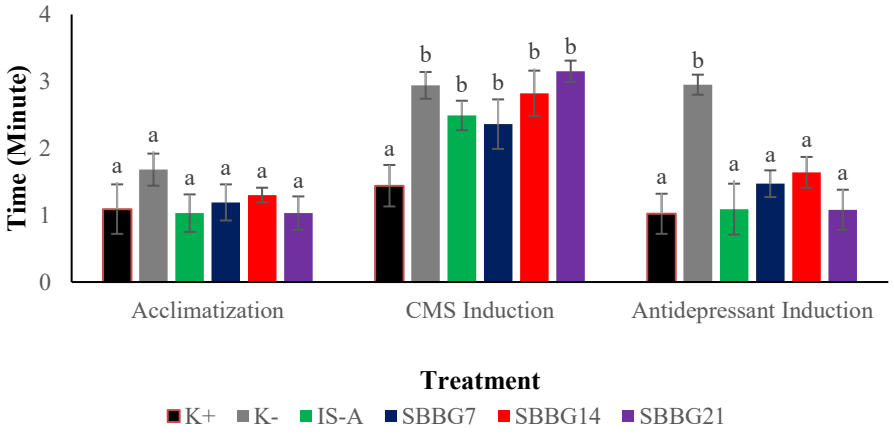

Figure 2. Duration of immobility in mice during acclimatization, chronic mild stress (CMS) induction, and antidepressant treatment phases. Values are presented as mean ± SE. C− = CMS control; C+ = normal control; IS-A = amitriptyline-treated group; SBBG7, SBBG14, and SBBG21 = Solo-bulb black garlic extract aged for 7, 14, and 21 days, respectively. Different superscript letters indicate significant differences among groups ($p < 0.05$).

Mice in the normal control group (C+) consistently exhibited low immobility duration, while chronic mild stress (CMS) significantly increased immobility in the negative control group (C−), confirming the successful induction of depression-like behavior. This elevated immobility persisted throughout the experimental period in the absence of intervention. In contrast, treatment with amitriptyline (IS-A) and solo-bulb black garlic extracts (SBBG7, SBBG14, and SBBG21) significantly reduced immobility duration compared to the untreated CMS group, indicating antidepressant-like effects (Figure 2). Among all treatment groups, SBBG21 produced the greatest reduction in immobility duration, approaching the levels observed in the IS-A and C+ groups.

Increased immobility in the Forced Swimming Test reflects behavioral despair associated with chronic stress and dysregulation of the hypothalamic–pituitary–adrenal axis and monoaminergic neurotransmission (Mikulska et al., 2021). The superior efficacy of SBBG21 may result from the enhanced accumulation of bioactive compounds during prolonged aging and fermentation, including polyphenols, flavonoids, alkaloids, tannins, steroids, and the organosulfur compound S-allylcysteine (SAC) (Chen et al., 2020). Phytochemical analysis demonstrated that SBBG21 possessed the highest total phenolic content (236.37 mg GAE/g), which is particularly relevant because phenolic compounds are major contributors to antioxidant capacity and are strongly positively correlated with antioxidant activity (Araújo et al., 2024). Prolonged aging has also been associated with increased antioxidant activity and neuroprotective effects, which may protect neuronal cells from oxidative damage associated with stress and depression (Astutiningsih et al., 2025). Additionally, SAC and other bioactive compounds may modulate central neurotransmitter systems, particularly serotonin, thereby contributing to mood regulation and alleviating depressive symptoms (Stępień et al., 2024).

Overall, these findings indicate that the antidepressant-like effects of SBBG21 are mediated by antioxidant, anti-inflammatory, and neuroprotective mechanisms, supporting the potential of whey kefir–fermented solo black garlic as a functional food-based complementary strategy for managing depression-related behavioral disturbances.

Swimming Activity Duration of Mice Induced by Solo-Bulb Black Garlic Extract

Swimming behavior in the Forced Swimming Test (FST) served as an indicator of adaptive coping and antidepressant-like activity, with increased swimming duration reflecting enhanced stress resilience and reduced depressive-like behavior. No significant differences were observed among groups at baseline, confirming similar initial conditions. After chronic mild stress (CMS) induction, the negative control group (C−) demonstrated a substantial reduction in swimming duration, supporting the detrimental impact of chronic stress on motivation and behavioral activity (Atrooz et al., 2021). In contrast, mice treated with amitriptyline (IS-A) and solo-bulb

black garlic extracts (SBBG7, SBBG14, and SBBG21) exhibited increased swimming duration compared with the C− group, indicating improved behavioral adaptation. SBBG21 produced the greatest increase in swimming duration, achieving levels similar to those observed in the amitriptyline-treated group. These results indicate that whey kefir–fermented solo black garlic, particularly after 21 days of aging, confers antidepressant-like effects by enhancing stress-coping behavior in CMS-induced mice.

Climbing Activity Duration of Mice Induced by Solo-Bulb Black Garlic Extract

In the Forced Swim Test (FST), increased climbing and swimming behaviors reflect enhanced motivation and noradrenergic activity. Amitriptyline, a tricyclic antidepressant, inhibits the reuptake of norepinephrine and serotonin, thereby elevating synaptic neurotransmitter levels and improving physical activity in stressed animals (Pereira et al., 2023).In this study, solo-bulb black garlic extract produced similar behavioral effects. During acclimatization, significant differences in climbing activity were observed among the groups. The normal control group (C+) exhibited the lowest climbing duration, while the groups treated with amitriptyline (IS-A) or solo-bulb black garlic extract (SBBG7, SBBG14, SBBG21) showed higher activity. This variation likely reflects differences in baseline responsiveness to novel environments. After chronic mild stress (CMS) induction, climbing duration decreased significantly in the untreated stress group (C-), indicating that prolonged stress impairs motivation and physical activity. Chronic HPA axis activation elevates cortisol, exerting catabolic effects on muscles and disrupting brain regions involved in motivation, thereby increasing immobility and reducing exploratory behavior (Figure 4). Conversely, stress-induced mice treated with amitriptyline or solo-bulb black garlic extract exhibited significantly higher climbing durations than untreated mice. Among the extract-treated groups, SBBG21 showed the greatest activity, approaching levels observed in the normal control and IS-A groups. These findings suggest that black garlic extract mitigates stress-induced behavioral deficits in a dose-dependent manner.

Phytochemical analysis revealed that solo black garlic contains polyphenols, flavonoids, alkaloids, tannins, and steroids, with phenolic compounds being most abundant in the 21-day aged extract (236.37 mg GAE/g). The antioxidant properties of phenolic compounds likely contribute to the extract's antidepressant-like effects by reducing oxidative stress, consistent with prior findings in plant-derived antidepressants (Araújo et al., 2024). Accordingly, 21-day-aged solo-bulb black garlic extract effectively alleviates CMS-induced behavioral deficits by enhancing climbing and swimming activity while reducing immobility. These results highlight its potential as a natural, alternative antidepressant therapy.

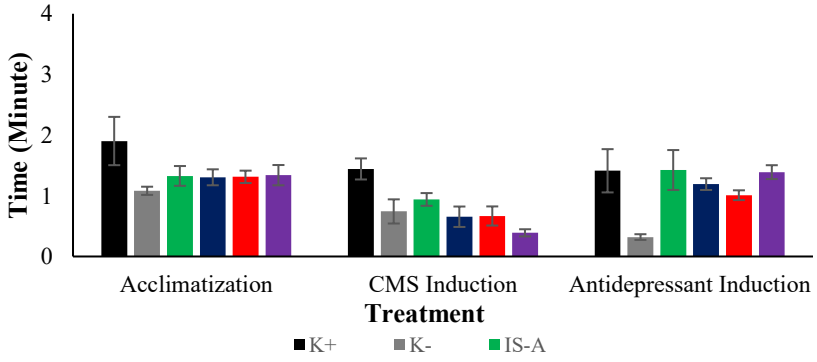

Figure 3. Swimming duration of mice during acclimatization, chronic mild stress (CMS) induction, and antidepressant treatment. Values are presented as mean ± SE. C− = CMS control; C+ = normal control; IS-A = amitriptyline-treated group; SBBG7, SBBG14, and SBBG21 = Solo-Bulb Black Garlic extract aged for 7, 14, and 21 days, respectively.

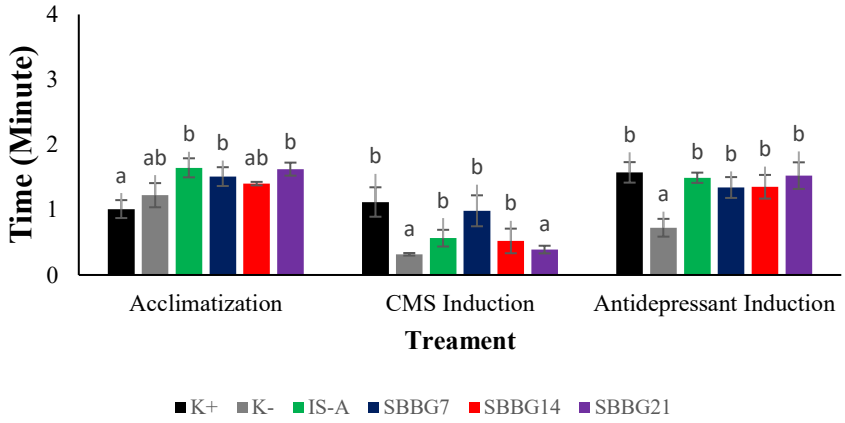

Figure 4. Climbing duration of mice during acclimatization, chronic mild stress (CMS) induction, and antidepressant treatment. Values are presented as mean ± SE. C− = CMS control; C+ = normal control; IS-A = amitriptyline-treated group; SBBG7, SBBG14, and SBBG21 = solo-bulb black garlic extract aged for 7, 14, and 21 days, respectively.

Table 3. Average and difference in body weight of mice (*Mus musculus*)

Treatment	Body Weight (g ± SE)			Δ1	Δ2
	Acclimatization	CMS Induction	Antidepressant Induction		
C+	32.00±0.35^{e}	34.00±0.37^{a}	36.00±0.33^{a}	2.00	2.00
C-	31.00±0.38^{de}	29.50±0.51^{b}	30.00±0.40^{c}	-1.50	0.50
IS-A	24.00±0.64^{a}	23.10±0.90^{bc}	31.00±0.59^{b}	-0.90	7.90
SBBG7	29.00±0.40^{cd}	28.65±0.61^{bc}	32.00±0.59^{b}	-0.35	3.35
SBBG14	27.00±0.57^{ab}	27.85±0.59^{cd}	32.00±0.98^{b}	0.85	4.15
SBBG21	28.00±0.53^{bc}	25.00±0.62^{d}	30.00±0.82^{bc}	-3.00	5.00

Note: C+ = normal control; C- = CMS control; IS-A = CMS + amitriptyline; SBBG7/14/21 = CMS + solo-bulb black garlic extract aged 7, 14, or 21 days. Different letters indicate significant differences ($p < 0.05$). Δ1 = acclimatization vs. CMS; Δ2 = CMS vs. antidepressant treatment. SE = Standard Error

Body Weight Gain Rate of Mice Induced by Solo-Bulb Black Garlic Extract

Body weight differed significantly among the treatment groups throughout the experimental periods. During the acclimatization phase, body weight remained stable in all groups, with mean values ranging from 24.00 to 32.00 g. Following CMS induction, the normal control group (C+) exhibited a significant increase in body weight, reflecting normal physiological growth in the absence of stress. In contrast, the negative control group (C−) showed a significant reduction in body weight, indicating the physiological impact of chronic stress exposure. After antidepressant administration, mice treated with amitriptyline (IS-A) and solo-bulb black garlic extracts (SBBG7, SBBG14, and SBBG21) demonstrated variable responses, with an overall trend toward increased body weight relative to the CMS induction period. Notably, the IS-A and SBBG7 groups showed significant recovery in body weight. Changes in body weight during the acclimatization, CMS induction, and antidepressant treatment periods are presented in Table 3.

Table 3 shows that mice treated with antidepressants, including amitriptyline (IS-A) and solo-bulb black garlic extract groups (SBBG7, SBBG14, and SBBG21), exhibited significant body weight recovery following the antidepressant induction period, with IS-A showing the greatest effect. Notably, SBBG aged for 14 and 21 days also produced marked improvements, supporting its antidepressant potential. These findings indicate that both chronic stress and antidepressant intervention significantly affect body weight regulation in mice. Chronic Mild Stress (CMS) induces sustained activation of the hypothalamic pituitary adrenal (HPA) axis, leading to elevated cortisol levels that disrupt metabolic homeostasis, suppress appetite, and promote catabolic processes, resulting in weight loss (Mosili et al., 2020). Stress-induced sympathetic activation further inhibits digestive activity and alters feeding behavior. In rodent models, chronic stress

is predominantly associated with reduced food intake rather than with stress-induced overeating. Stress-related sleep disturbances may also contribute to appetite dysregulation through altered ghrelin and leptin signaling. Following CMS induction, mice treated with IS-A and SBBG showed significantly greater body weight gain than the negative control group. Amitriptyline enhances serotonergic and noradrenergic signaling, improving stress-related behaviors and appetite regulation, while its sedative effects may normalize sleep and reduce cortisol levels (Saryono et al., 2023). Similarly, solo-bulb black garlic extract may mitigate stress-induced metabolic disturbances, thereby facilitating recovery of body weight, particularly at longer aging durations.

Leukocyte and Erythrocyte Responses to Whey Kefir–Fermented Solo-Bulb Black Garlic in CMS Mice

Based on the observation, CMS induction altered leukocyte and erythrocyte counts compared with the normal control group, while antidepressant administration, including amitriptyline and solo-bulb black garlic extracts, resulted in different hematological values among treatment groups (Table 4).

Table 4. Effects of antidepressant treatments on leukocyte and erythrocyte levels in CMS-induced mice. Values are expressed as mean ± SE. C+ = normal control; C− = CMS control; IS-A = amitriptyline-treated group; SBBG7, SBBG14, and SBBG21 = Solo-Bulb Black Garlic extract aged for 7, 14, and 21 days, respectively. Different superscript letters within the same column indicate significant differences ($p < 0.05$).

No	Treatment	Leukocyte (10^3/µL ±SE)	Erythrocyte (10^6 /µL ±SE)
1	C+	7.96±0.94^{a}	7.08±0.83^{a}
2	C-	3.98±0.47^{b}	3.98±0,31^{b}
3	IS-A	7.19±0.80^{a}	6.72±0.81^{ab}
4	SBBG7	5.01±0.30^{ab}	5.23±0.50^{ab}
5	SBBG14	7.08±0.93^{ab}	6.87±0.42^{ab}
6	SBBG21	7.89±0.49^{a}	6.19±0.81^{ab}
Leukocyte standard (McClure, 2017).		6-15 × 10^3 /µl	
Erythrocyte standard (McClure, 2017).			7-12.5 × 106 µL

One-way ANOVA showed significant differences in leukocyte ($F(5.18) = 5.489$; $p = 0.003$) and erythrocyte counts ($F(5.18) = 3.395$; $p = 0.025$) among the treatment groups. CMS exposure significantly reduced both parameters in the negative control (C−) compared with the normal control (C+), indicating stress-induced hematological alterations. Duncan's post hoc test showed that treatment with amitriptyline (IS-A) and solo-bulb black garlic extract treatments (SBBG7, SBBG14, SBBG21) tended to restore leukocyte counts toward normal levels. Erythrocyte counts in the C− group were below the physiological range, whereas all treated groups maintained values within normal limits. These results suggest that solo-bulb black garlic extract may alleviate CMS-induced hematological disturbances. Stress induction significantly reduced leukocyte and erythrocyte counts in mice, indicating a systemic hematological response to chronic stress.

Treatment with amitriptyline and solo-bulb black garlic extract partially attenuated these effects, suggesting protection against stress-induced immune and erythropoietic suppression, although not all treatments reached statistical significance. Chronic stress activates the hypothalamic–pituitary–adrenal (HPA) axis, resulting in sustained cortisol release that suppresses leukocyte production, promotes immune cell apoptosis, and disrupts erythropoietin-mediated erythropoiesis (Barrett et al., 2021). Prolonged neuroendocrine activation also increases oxidative stress, leading to reactive oxygen species–mediated damage to erythrocyte membranes and hemoglobin, thereby reducing red blood cell stability and function (Mosili et al., 2020). The tendency of SBBG extract to restore blood cell counts may be attributed to its antioxidant and anti-inflammatory properties, which help maintain hematological homeostasis under chronic stress conditions.

Oxidative stress induced by chronic stress damages erythrocyte membranes, increasing permeability and susceptibility to hemolysis. Damaged red blood cells are removed by the reticuloendothelial system, particularly the spleen, and severe oxidative conditions may trigger eryptosis, leading to reduced erythrocyte counts and impaired oxygen transport (Obeagu et al., 2024). Consequently, this reduction can result in tissue hypoxia and systemic dysfunction. Amitriptyline, a tricyclic antidepressant, modulates stress responses by regulating monoamine neurotransmission and HPA axis activity, which may indirectly influence leukocyte and erythrocyte homeostasis, although its hematological effects are variable. In contrast,

solo-bulb black garlic extract appears to exert protective effects through its antioxidant and anti-inflammatory properties. Bioactive compounds, such as S-allyl cysteine and polyphenols, may reduce reactive oxygen species production, suppress pro-inflammatory cytokines, and support hematopoietic activity in the bone marrow, thereby contributing to the restoration of leukocyte and erythrocyte levels under chronic stress. By attenuating oxidative and inflammatory signaling, solo-bulb black garlic extract may also reduce excessive HPA axis activation, thereby supporting immune and hematological recovery in stressed mice (Obeagu et al., 2024).

Organoleptic Characteristics of Whey Kefir Fermented Single Clove Garlic

Organoleptic evaluation was conducted to determine the preference of 46 panelists for SBBG fermented with whey kefir at varying aging periods. The testing was conducted using a hedonic test with a Likert scale of 1-5. Parameters observed included color, aroma, taste, texture, and overall preference (Table 5).

Table 5. Average organoleptic scores of whey kefir–fermented SBBG at different aging times

Parameters	Organoleptic Value		
	SBBG-7	SBBG-14	SBBG-21
Color	3.04 ± 0.87[a]	3.26 ± 0.80[ab]	3.54 ± 1.29[b]
Aroma	3.48 ± 1.07[a]	3.11 ± 1.08[a]	3.17 ± 1.29[a]
Taste	2.75 ± 1.04[a]	2.93 ± 1.12[a]	3.00 ± 1.28[a]
Texture	2.91 ± 1.03[a]	3.35 ± 1.08[a]	3.35 ± 1.08[a]
Overall preference	3.07 ± 0.88[a]	3.13 ± 0.98[a]	3.20 ± 1.11[a]

Score category: 1 = dislike very much, 2 = dislike, 3 = somewhat like, 4 = like, 5 = like very much

Color

Color was visually evaluated by panelists as a physical parameter. The average preference scores for whey kefir–fermented Solo-Bulb Black Garlic (SBBG) at different aging periods ranged from 3.04 to 3.54, corresponding to a "somewhat like" category. The highest score was obtained by SBBG-21 (3.54), indicating that longer aging times produced a darker color with greater visual appeal. The overall color preference followed the order SBBG-7 < SBBG-14 < SBBG-21 (Figure 5). These color changes are attributed to non-enzymatic Maillard reactions during aging, which generate melanoidin compounds responsible for the characteristic brown color and flavor of SBBG.

Aroma

Fresh Solo-Bulb Black Garlic has a pungent aroma due to the presence of volatile organosulfur compounds such as allicin, diallyl disulfide (DADS), diallyl trisulfide (DATS), and allyl methyl sulfide (AMS). These compounds are formed when bulb tissue is damaged, and the enzyme allinase catalyzes the conversion of allicin to allicin, which subsequently decomposes into volatile organosulfur derivatives. These compounds undergo a compositional change during the aging process, resulting in the loss of the pungent aroma SBBG. Aging also promotes the formation of new volatile compounds, which give SBBG its distinctive aroma. Furthermore, during the aging process, the Maillard reaction produces melanoidin compounds, which contribute to its distinctive and complex aroma (Yang et al., 2019). Pretreatment by whey kefir fermentation also enriches the aroma profile through the activity of lactic acid bacteria, which produce lactic acid, acetaldehyde, and diacetyl, imparting a mild sourness.

(a) (b) (c) (d) (e)

Figure 5. Color changes of solo black garlic during whey kefir fermentation and aging:(a) fresh single garlic, (b) whey kefir–fermented garlic for 7 days, (c) Solo-Bulb Black Garlic aged 7 days, (d) Solo-Bulb Black Garlic aged 14 days, and (e) Solo-Bulb Black Garlic aged 21 days.

Taste

The whey kefir-fermented SBBG was most preferred by panelists with an average score of 3.00 (somewhat liked), while the least preferred was SBBG-7, with an average score of 2.74 (disliked). These results indicate that longer aging time improved the panelists' acceptance of the taste of whey kefir-fermented SBBG. The results showed that the 7-day SBBG still had a distinctive bitter garlic flavor and was slightly bland, while the 21-day SBBG tended to be sweeter and slightly sour. These changes are likely attributable to the fermentation process and the formation of melanoidin compounds during the Maillard reaction, which contribute to SBBG's distinctive flavor. This finding aligns with research by Nam et al. (2023), who found that garlic develops a sweeter taste with increasing aging time due to the formation of melanoidin compounds during the Maillard reaction.

Texture

The organoleptic evaluation showed differences in the texture of whey kefir-fermented solo black garlic (SBBG) at different aging periods, particularly for SBBG-7. After 7 days of aging (SBBG-7), panelists perceived SBBG-7 as having a relatively firm texture. This is likely because of the Maillard reaction that had not progressed sufficiently to soften the tissue structure. These results align with texture measurements obtained using a fruit hardness tester, which demonstrated the effect of aging time on the texture of SBBG fermented with whey kefir. Texture is an important parameter in food products because it can influence consumer acceptance. Leng et al. (2020) reported that aging temperatures above 50°C hydrolyze cell wall polysaccharides, thereby reducing garlic firmness. Similarly, Kang (2016) noted that pH reduction during aging contributes to polysaccharide degradation, producing a soft and chewy texture in SBBG. Consistent with these findings, SBBG-21 exhibited the lowest pH (6.0) and a chewy texture, which was preferred by panelists.

In general, panelists preferred SBBG with longer aging periods. SBBG-21 received the highest scores for color (3.54 ±1.29, "somewhat liked"), texture (3.35±1.08), and overall preference (3.20±1.11), reflecting the dark brown appearance and chewy texture characteristic of aged SBBG. The color change is attributed to melanoidin formation during the Maillard reaction. These results indicate that prolonged aging enhances the sensory appeal of SBBG. SBBG-7 obtained the lowest average value of 3.07, and SBBG-21 obtained the highest average value of 3.2, likely due to changes in its color, aroma, taste, and texture during the aging process. This indicates that longer aging time produces SBBG with the most desirable organoleptic qualities.

The novelty of this study lies in the application of whey kefir as a pre-fermentation medium before the aging of single clove garlic. Previous studies have mainly focused on conventional thermal aging or alternative fermentation media, whereas the use of whey kefir in this context remains limited. The incorporation of whey kefir introduces probiotics and bioactive compounds that may enhance the functional properties of the final product. In addition, this study evaluates the effect of different aging periods (7, 14, and 21 days), providing insight into process optimization. This combined approach offers a new strategy to improve the antioxidant and antidepressant potential of single clove garlic.

This study contributes to scientific development by providing evidence that fermented functional foods can influence physiological and behavioral responses associated with depression. The results support previous findings that oxidative stress and inflammation play important roles in the pathophysiology of depression (Mikulska et al., 2021). By reducing these factors, bioactive compounds in Solo-Bulb Black Garlic may help improve neurobiological function. From a societal perspective, this research also offers a natural and potentially safer alternative to conventional antidepressants, which are often associated with adverse effects (Braund et al., 2021). Thus, this study supports the development of food-based approaches to improve mental health and quality of life.

This research supports SDG 3 (Good Health and Well-being) by contributing to the development of alternative strategies for managing depression through functional foods. Mental health disorders remain a major global health concern, and innovative approaches are required to address this issue (UN DESA, 2025). In addition, this study supports SDG 12 (Responsible Consumption and Production) by promoting value-added utilisation of agricultural products such as garlic. Fermentation and aging processes can enhance the nutritional and functional value of food products, thereby reducing waste and increasing sustainability (UN DESA, 2025). Therefore, this research aligns with global efforts to improve both public health and

sustainable food systems.

CONCLUSION

Different aging periods significantly influenced the antidepressant activity of solo black garlic fermented with whey kefir in male mice (*Mus musculus*) subjected to chronic mild stress (CMS). Among the tested groups, the 21-day-aged solo black garlic (SBBG-21) fermented with whey kefir showed the greatest antidepressant effect. The SBBG21 group exhibited the greatest reduction in immobility duration (1.08 ± 0.30 min), with increases in swimming (1.39±0.11 min) and climbing (1.53±0.20 min). Leukocyte (7.89 ± 0.49×10^3 cells/μL) and erythrocyte (6.19 ± 0.81×10^6 cells/μL) counts were close to normal. Organoleptic scores for SBBG21 were highest for color (3.54 ±1.29), aroma (3.17±1.29), taste (3.00±1.28), texture (3.35 ±1.08), and overall preference (3.20±1.11), reflecting improved sensory quality with longer aging periods. These results indicate that whey kefir–fermented SBBG aged for 21 days combines both antidepressant efficacy and favorable sensory attributes, supporting its potential as a natural functional food for depression management. This study is limited by the lack of investigation into the underlying biochemical and molecular mechanisms, such as neurotransmitter activity and inflammatory responses, that may explain the observed antidepressant effects. Future research should focus on elucidating these mechanisms, particularly the regulation of neurotransmitters, brain-derived neurotrophic factor (BDNF), and the hypothalamic-pituitary-adrenal (HPA) axis.

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AUTHOR CONTRIBUTION STATEMENT

All authors contributed significantly to the work reported in this manuscript. RAU designed the research, MU wrote the manuscript, and assisted with its submission. MDN and NFP collected and analyzed the data. EY provided critical feedback and reviewed the manuscript for grammatical accuracy. All authors read and approved the final manuscript and agreed to be accountable for all aspects of the work.

INFORMED CONSENT STATEMENT

All participants provided written informed consent before the study. Ethics Committee of Universitas Padjadjaran, Bandung, Indonesia (Ethical Approval No. 774/UN6.KEP/EC/2025; Registration No. 2507020818). This approval ensured that all measures were taken to minimize animal suffering and to use the minimum number of animals required to achieve valid scientific results.

CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declare that no artificial intelligence (AI) tools were used in the generation, analysis, or writing of this manuscript. All aspects of the research, including data collection, interpretation, and manuscript preparation, were carried out entirely by the authors without the assistance of AI-based technologies.

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