Enhancement of antioxidant activity and bioactive compounds in soy whey fermented with *Lactiplantibacillus plantarum* and *Weissella confusa*

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Abstract

Soy whey (SW) is a byproduct from tofu and soy protein isolate (SPI) production that contains various nutrients such as protein, amino acids, minerals, carbohydrates, isoflavones. In this study, SW was fermented with lactic acid bacteria (LAB) with the aim to enhance total phenolic contents (TPC), Gamma amino butyric acid (GABA) and antioxidant activity. At first, eight different LAB strains were screened, and then the activity and cell counts of the most potent strains were investigated during fermentation. The results showed that all the isolates were able to grow in SW and the increase in incubation time significantly ($p<0.05$) decreased the pH of all samples from 5.75 to 4.5. Among eight LAB isolates, *lactiplantibacillus plantarum* MCM4 and *weissella confusa* MDM8 showed higher activity in terms of acid production, increase in TPC content and proteolytic activity. The sample fermented by *L. plantarum* MCM4 had the highest content of free amino acids (1.73 mg/ml) and the unfermented sample with 0.9 mg/ml had the lowest content. GABA concentration varied from 6.15 mg/mL (unfermented) to 24.175 mg/100 mL (SW fermented with *L. plantarum* MCM4).

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In this research, it was found that fermentation increased the antioxidant capacity of SW in such a way that the highest amount was observed in the fermented sample with *lactiplantibacillus plantarum* MCM4. A positive correlation (R^2= +0.72) was found between viable cell counts and proteolysis. It can be concluded that, fermentation with *L. plantarum* MCM4 and *W. confusa* MDM8 can be applied as an approach to valorize SW.

Keywords: Biorefinery, Gamma aminobutyric acid, Lactic Acid Bacteria, Fermentation

Introduction

Soy whey (SW) is a by-product from Tufo cheese and soy protein isolate (SPI) production that contains various nutrients such as proteins, amino acids, carbohydrates, isoflavones and Gamma-aminobutyric acid (GABA) (Belén et al., 2013). Nine kilograms of SW is produced from 1 kg soy used for Tufo production, and 20 tonnes of SW is produced per 1 ton of SPI. SW nutrients can be used for the production of functional beverages, bioactive peptides, enzymes and biogas. Because of its high BOD and COD, SW disposal as a waste cause environmental problems (Wang & Ying, 2007). Therefore, it needs further treatments before disposal which is an expensive operation. Because of the lack of appropriate technology and enough economic motivation for SW recycling, the most of produced SW is disposed as the waste water that cause environmental challenges and water contamination (Candow, Burke, Smith-Palmer, & Burke, 2006). Regarding to the production of high amounts of SW from Tufo and SPI processing, it is essential to find a solution for its valorization and efficient management (Chua & Liu, 2019).

The use of agricultural waste and recycling the industrial by-products have gained much attention during the recent decades. In this regard, bio-refinery is referred to the bioconversion

of agricultural and industrial wastes to the value-added products by using biological factors (Kumar et al., 2022). Among the methods applied for the waste valorization, microbial fermentation has a unique place as it may result in the formation of health-promoting compounds. Microbial fermentation has been widely used for the valorization of cheese whey into fermented and alcoholic beverages. In the bio-refinery projects, lactic acid bacteria (LAB) are of great importance mainly because of their safety and adaptation to various ecosystems.

When grown in a nutrient media, (LAB) synthesize low-molecular weight compounds that contribute to the improvement of aroma and sensorial properties of the final product (König, Unden, & Fröhlich, 2009). Lactic fermentation can be applied for the valorization of SW to high value-added products, or recycling its nutrients. Recently, alcoholic beverages have been produced from SW using *saccharomyces* and non-*saccharomyces* yeasts (Chua, Lu, & Liu, 2017, 2018). In addition, a SW-based beverage has been developed using *Lactobacillus plantarum* B1-6 (Xiao et al., 2015) and *Lactobacillus amylolyticus* L-6 (Fei et al., 2017). The recent study by Tu et al., (2019) has shown that SW can be fermented to a functional Kombucha (Tu, Tang, Azi, Hu, & Dong, 2019). However, compared to the cheese whey, few studies have been done on SW fermentation. In the current study, the effects of proteolytic LAB fermentation on the antioxidant activity and bioactive compounds of SW have been investigated.

Materials and Methods

Materials and Microbial Cultures

SW used in this study was obtained from Donya factory (Golestan province, Iran). The proteolytic LAB used in this study (MDM8, MDM21, MCM4, BRM3, SRM2, ORT2, ORM4,

ORM3) had been previously isolated from pickled cabbage, sour dough and raw milk (Table 1) (Karimian, Moayedi, Khomeiri, Aalami, & Mahoonak, 2020; Khanlari, Moayedi, Ebrahimi, Khomeiri, & Sadeghi, 2021; Moayedi, Mahmoudi, Khomeiri, & Loghman, 2019). All the proteolytic LAB used in this study had been kept as frozen cultures in the microbial bank.

Table1. LAB strains used in this study

Code	Similarity (%)	Name (NCBI)	Source	Reference
MCM4	98.4	*Lactiplantibacillus plantarum*	Pickled cabbage	Karimian *et al*. (2020)
MDM8	98.8	*Weissella confusa*	sourdough	Khanlari et al. (2021)
MDM21	99.2	*Entrococcus faecium*	sourdough	Khanlari et al. (2021)
BRM3	99	*Lactococcus lactis* subsp. *lactis*	Raw milk	Moayedi et al. (2019)
SRM2	99	*Lactobacillus reuteri*	Raw milk	Moayedi et al. (2019)
ORT2	98	*Lactobacillus delbrueckii*	Raw milk	Moayedi et al. (2019)
ORM3	97	*L. delbrueckii* subsp. *bulgaricus*	Raw milk	Moayedi et al. (2019)
ORM4	99	*Lactobacillus curvatus*	Raw milk	Moayedi et al. (2019)

Screening of bacterial isolates in terms of acidifying activity in SW

For the determination of acidifying capacity of bacterial cultures, SW was sterilized at 108 °C for 15 min in an autoclave. Then it was inoculated (2%, v/v) with each bacterial culture (turbidity around 0.25 at 600 nm) and incubated at 37 °C. The pH value of incubated samples were measured at time intervals 0, 12, 24 and 48 h.

Viable cell counts during fermentation

Viable cell counts of bacterial isolates added to the media was determined using pour plate (at 0, 12, 24 and 36 h) method as described by (Gül, Özçelik, Sağdıç, & Certel, 2005). Two serial dilution was used and the cell counts was determined according to the following formula (Moslemi, Moayedi, Khomeiri, & Maghsoudlou, 2023):

$$N = \frac{\sum Ci}{V(n1 + 0.1n2)d}$$

Where $\sum Ci$ is total colony counted, V volume, n1 the cell counts from the first dilution, n2 the cell counts in the second dilution, and d the least dilution used for cell counts determination.

Determination of free amino acid content (proteolytic activity)

The method previously described by Church et al. (1983) was used for the determination of proteolytic activity. In this method, amino groups reacted with OPA that results in increase in absorbance at 340 nm. A standard curve was prepared using L-Serine as the standard amino acid (Church, Swaisgood, Porter, & Catignani, 1983).

Determination of total phenolic compounds (TPC)

The TPC was determined by using Foline Ciacalteau method (Xiao et al., 2015). The results were expressed as mg of Gallic acid equivalents per ml (mg GAE/ml).

Selection of the potent isolates to achieve the maximum bioactivity

After the screening, LAB with the highest capacity to increase TPC and proteolytic activity were selected to achieve the highest bioactivity of fermented SW. The type of bacterial culture and fermentation time were considered as the variables, while DPPH scavenging activity, TPC, GABA content, total antioxidant capacity and ferric reducing antioxidant potential (FRAP) were the responses.

Determination of GABA concentration

GABA concentration was determined using high performance liquid chromatography equipped with UV detector as reported by Karimian et al. (2020). The filtrate containing GABA was derivatized with phenylisothiocyanate (PITC) followed by detection at 254 nm (Karimian et al., 2020).

Determination of DPPH scavenging activity, FRAP and TAC

For the determination of DPPH scavenging activity, 650 µm of fermented sample was added to 1000 µm deionized water and 1000 µm DPPH solution (0.15 mM) and kept at a dark place (room temperature) for 20 min. Then the absorbance was read at 517 nm using UV-Visible spectrophotometer. Deionized water was used as the blank and DPPH scavenging activity was calculated according to following equation:

$$DPPH\ scavenging\ activity\ (\%) = (\mathrm{Ac} - \frac{\mathrm{As}}{\mathrm{Ac}}) \times 100$$

Where Ac and As were the absorbance for the sample and control (DPPH solution), respectively.

FRAP was evaluated similar to method previously described by (Yıldırım, Uğur, & Kutlu, 2017). TAC was determined according to the method of Meshginfar et al., 2018 with slight modification. For the preparation of TAC solution, 3.25 ml H_2SO_4 (0.6 M), 1.064 g Na_2SO_4 and 0.49 g ammonium molibdate were mixed and reached to 125 ml. 15 µl of each sample was added to 1 mL of TAC solution and incubated at 90 °C for 60 min. After cooling, the absorbance was read at 695 nm. Deionized water was used as the blanc and ascorbic acid as the standard (Meshginfar, Sadeghi Mahoonak, Hosseinian, Ghorbani, & Tsopmo, 2018).

Statistical analysis

Statistical analysis was performed with factorial experiments (completely randomized design) using SAS software. The mean values were compared to each other using Duncan's multiple range test (95 % confidence interval).

Results and Discussion

Bacterial culture screening

The acidifying capacity of eight different LAB isolates (BRM3, MDM21, MCM4, SRM2, MDM8, ORM3, ORM4 and ORT2) in SW was investigated at different time intervals (0, 4, 12 and 24 h) of incubation (Fig. 1). All the tested isolates had the ability to grow in SW that resulted in reduction in pH from 5.75 to 4.5. The effects of incubation time and isolates were significant on pH changes ($P \leq 0.05$). Similar results have been reported on the growth ability of LAB strains in soy milk (Xu et al., 2019) and the media containing soy protein isolate (Yang, Ke, & Li, 2021) followed by pH reduction.

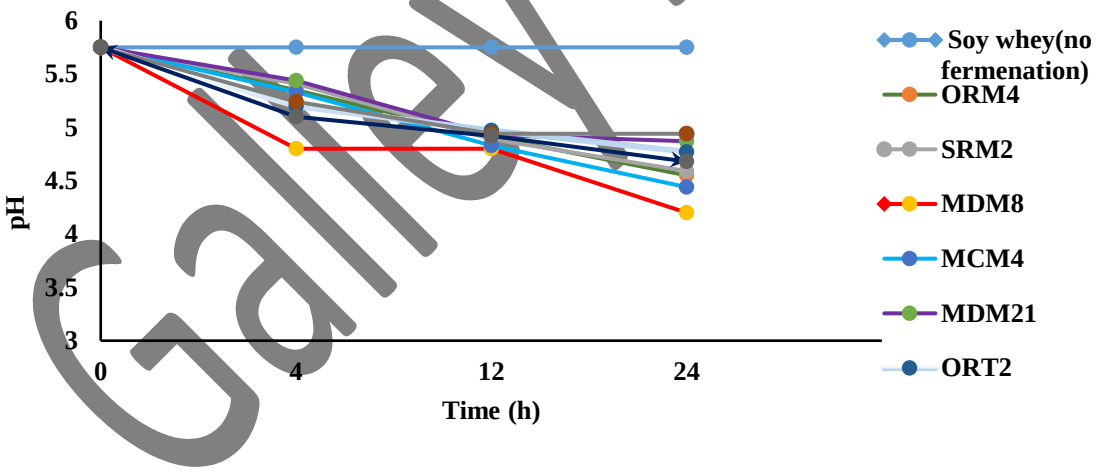


Figure 1- pH and acidity of soy whey fermented by different LAB isolates

Total phenolic compounds (TPC)

The results of TPC change in SW after 24 h of fermentation are shown in Fig. 2. Fermentation caused significant changes in TPC of all fermented sample, and there was significant difference among various LAB tested. The lowest TPC content was found in unfermented SW (0.49 mg GAE/ml) and the highest TPC in the sample fermented with *Weissella confusa* MDM8 (1.27 mg GAE/ml). It has been reported that beta-glucosidase produced by LAB during fermentation is responsible for increase in TPC (Lee, Hung, & Chou, 2008). Moreover, some phenolic compounds in insoluble fibers may be released as affected by fermentation (Chandrasekara & Shahidi, 2012). Phenolic compounds have different biological activities such as antioxidant, anticancer, antibacterial, anti-atherosclerosis, and anti-carcinogenic effects (Chung, Seo, Ahn, & Kim, 2011).

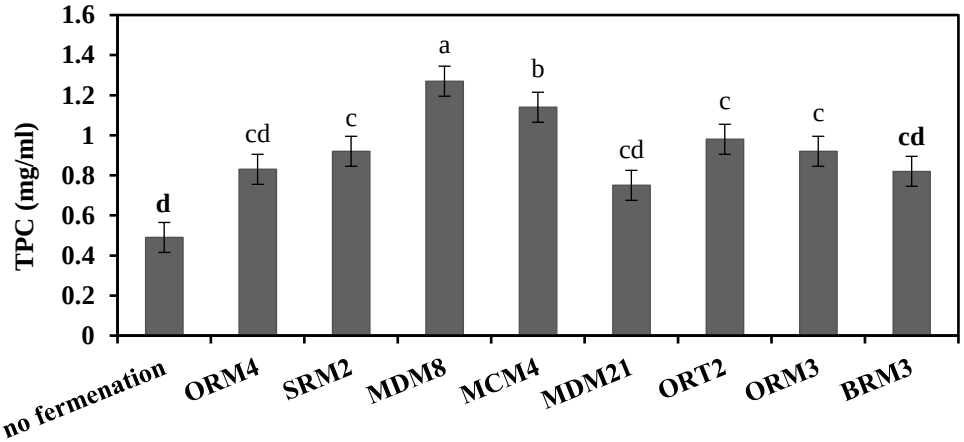


Figure 2- Variations in the total phenolic content (TPC) of fermented soy whey (after 24 hours) by eight lactic acid bacteria isolates.

Different letters on each column indicate significant differences between the samples ($p < 0.05$).

Free Amino Acid (Proteolysis)

As shown in Fig. 3, the type of inoculated LAB had significant effects on free amino acid content in fermented SW. SW fermented by *Lactiplantibacillus plantarum* MCM4 contained the highest FAA content (1.73 mg/ml), while unfermented SW contained the lowest content (0.9 mg/ml). It is clear that LAB tested in this study had the affinity to soy proteins which resulted in protein degradation into small peptides and free amino acids (Sharma, Garg, Kumar, Bhatia, & Kulshrestha, 2020). FAA content has been shown to be increased in parallel with increase in fermentation time (Baumann & Bisping, 1995; Bekiroglu et al., 2023). In addition, it has been reported that in tempe fermentation, bacteria with high proteolytic activity release amino acids five times higher than others, and such activity is affected by relative humidity and fermentation temperature (Baumann & Bisping, 1995). Peptides released during fermentation may have various functional activity such as antioxidant, antihypertensive, antibacterial, anticancer, anti-diabetic activities (Li & Wang, 2021).

According to the results obtained from screening the tested LAB in SW in terms of acidifying activity (Fig. 1), effects on TPC, and proteolysis, *L. plantarum* MCM4 and *W. confusa* MDM8 showed better activity and were selected for further investigations.

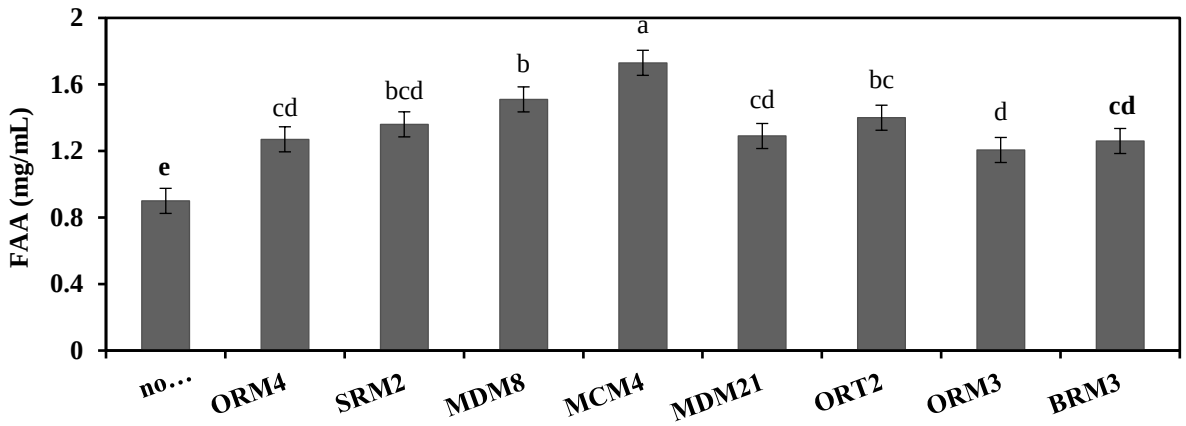


Figure 3- The content of free amino group in soy whey fermented by 8 isolates of lactic acid bacteria during 24 hours of fermentation. Different letters on each column indicate significant differences between samples ($p<0.05$).

Effects of fermentation with selected strains on bioactivity and bioactive compounds of SW

Cell Counts

Changes in viable cell counts of *L. plantarum* MCM4 and *W. confusa* MDM8 during SW fermentation are shown in Fig. 4. Both tested LAB strains grew well in SW, and cell counts increased as fermentation time increased to 24 h (Fig. 4-a), however it remained constant after 24 h. During soy fermentation, cell counts of LAB increased significantly ($p<0.05$) when fermentation time increased from 24 h to 36 h, and then it became constant (Zhang et al., 2014). In another study, all tested LAB isolates entered stationary phase at after 12 to 18 h fermentation of soy milk (Undhad Trupti, Das, Solanki, Kinariwala, & Hati, 2021). In addition, Gan et al. (2017) stated that cell counts of *L. plantarum* increased markedly during 9 h of fermentation in soy milk (Gan, Shah, Wang, Lui, & Corke, 2017). In the initial stages of fermentation, an increase in bacterial cell counts is observed due to the presence of fermentable raw materials and desirable conditions. When fermentation time is extended, viable cell counts will decrease because of undesirable conditions such as oxygen reduction and enhanced acidity (Liu et al., 2021).

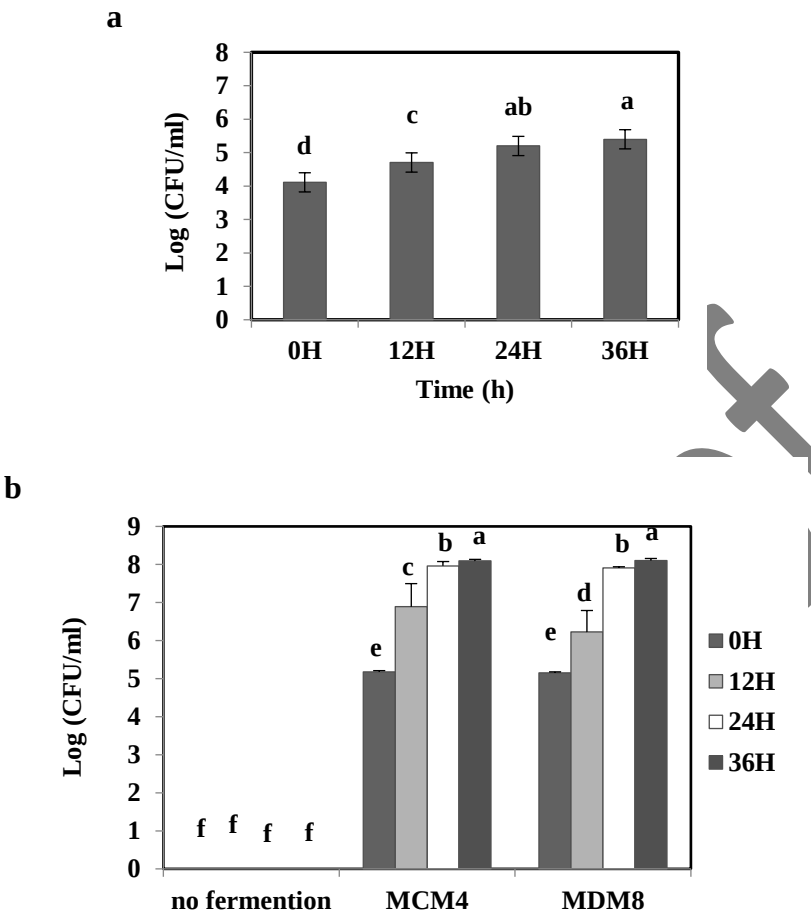


Figure 4- Cell count in soy whey fermented by 2 isolates of *Lactiplantibacillus plantarum* MCM4 and *Weissella confusa* MDM8 (a) main effect of time and (b) interaction effect of isolate type and time on cell counts. Different letters on each column indicate significant differences between samples ($p<0.05$).

Effect of fermentation on free amino acid content (proteolysis)

Proteolytic activity of LAB has been widely studied because of its industrial importance and essential role in bacterial (Lim, Foo, Loh, Mohamad, & Abdullah, 2019). In the current study, proteolytic activity of two LAB strains was investigated in SW during 36 h incubation at 37

°C (Fig. 5). It was found that FAA content is affected by LAB strains, as the highest proteolysis was observed in the sample fermented by *W. confusa* MDM8 (0.9 mg/ml) and the lowest amount in unfermented sample (0.7 mg/ml). As can be seen in Fig. 5-a, fermentation longer than 24 h, did not increase proteolysis in the sample fermented with *L. plantarum* MCM4, while there was no significant difference between the samples fermented with *W. confusa* MDM8 after 12, 24 and 36 h of incubation. In the previous studies it has been reported that there was a positive correlation between fermentation time and FAA content (Baumann & Bisping, 1995; Bekiroglu et al., 2023).

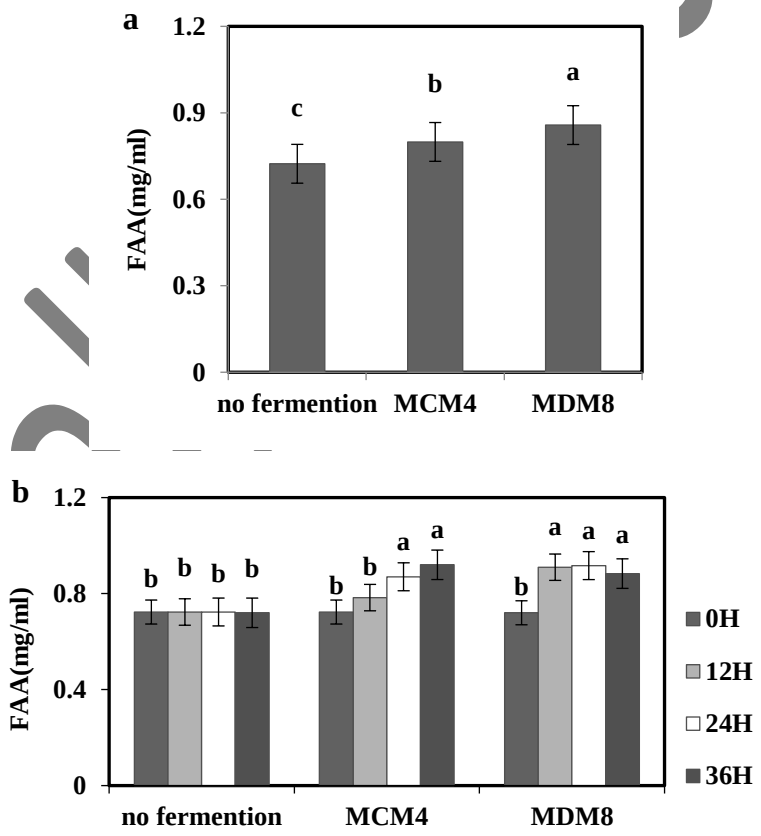


Figure 5- Free amino acid (FAA) content in soy whey fermented with *L. plantarum* MCM4 and *W. confusa* MDM8. (a) The main effect of bacteria and (b) the interaction

effect of bacteria and time on free amino group content. Different letters on each column indicate significant differences among the samples (p<0.05).

Effect of fermentation on total phenolic compounds (TPC)

The TPC of the samples is shown in Fig 6. It is observed that the phenolic compound content significantly increases with the fermentation time ($p < 0.05$), as the highest TPC was observed in the sample fermented by *L. plantarum* MCM4 (1 mg GAE/ ml) and the lowest amount in unfermented sample (0.7 mg GAE/ ml). It has been reported that fermenting soy with various microorganisms, including *Lactiplanti-bacillus plantarum*, leads to an increase in phenolic compound content (Fernandez-Orozco et al., 2007). Additionally, an increase in phenolic and flavonoid content in soy flour fermented with *Lactobacillus casei* has been reported (S. Li et al., 2020).

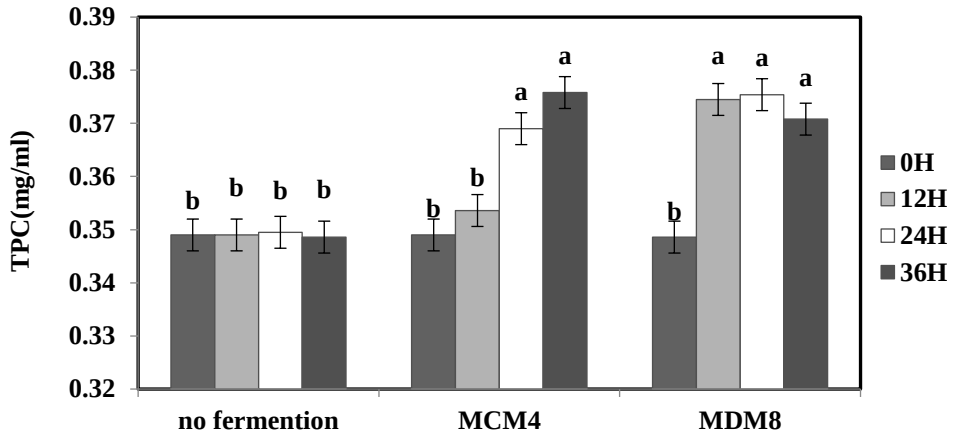
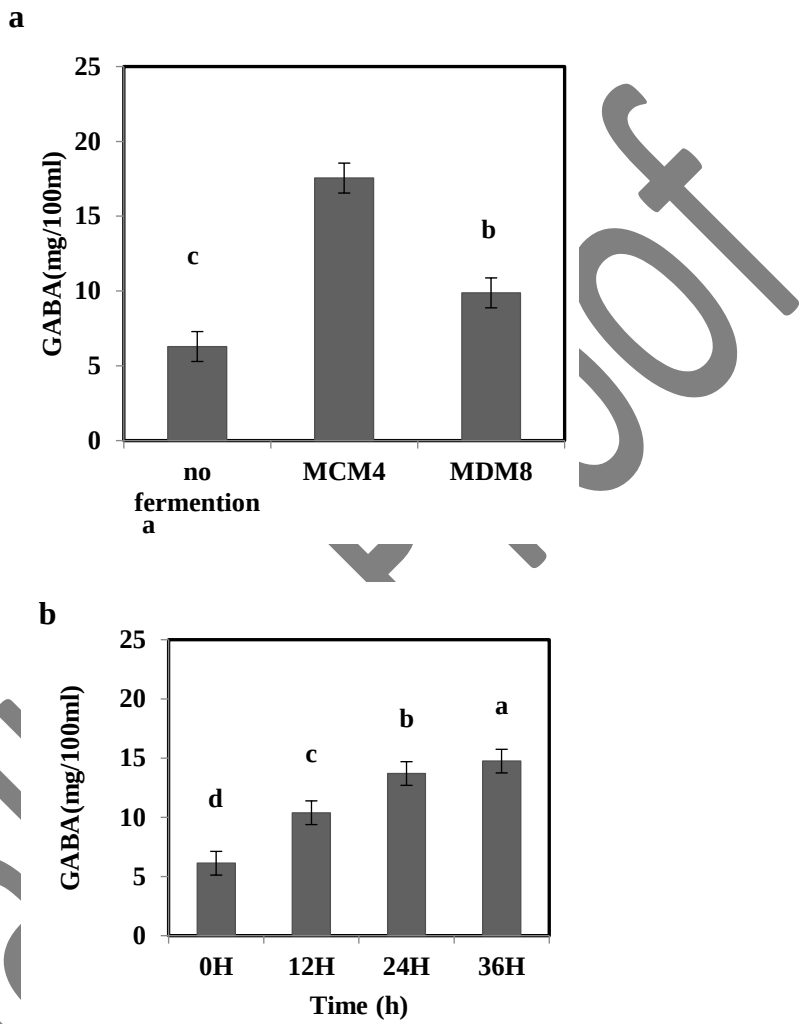


Figure 6- The phenolic compound content in soy whey fermented with *L. plantarum* MCM4 and *W. confusa* MDM8 during fermentation.
Different letters on each column indicate significant differences between the samples ($p < 0.05$).

Effect of fermentation on GABA content

GABA is a non-protein amino acid with a wide distribution in the nature that has been comprehensively studied because of its various physico-chemical functions and its positive effects on metabolic disorders (Pannerchelvan, Rios-Solis, et al., 2023). Among the organisms, LAB are one the most important GABA producers, and therefore the processes in which LAB strains are applied are highly considered (Pannerchelvan, Muhamad, et al., 2023). In this study, all the samples contained GABA, and its concentration varied from 6.5 mg/ml (unfermented SW) to 24.18 mg/ml (SW fermented with *L. plantarum* MCM4) (Fig. 7). As shown in Fig. 7-a, there was significant differences between *L. plantarum* MCM4 and *W. confuse* MDM8 in terms of their ability in GABA synthesis. Moreover, it was found that fermentation time had significant effect on GABA content, as it was increased when fermentation time increased (Fig. 7-b). This can be attributed to increase in viable cell counts, and subsequently increase in bioconversion of glutamic acid to GABA (Moayedi, Zareie, Yaghoubi, & Khomeiri, 2022). GABA concentration in the fermented foods may be influenced by different factors such as pH, temperature, media composition (for example GABA precursors) and inoculation volume (Khanlari et al., 2021). Aoki et al. (2013) reported that GABA content in fermented soy increased with increase in fermentation time (Aoki et al., 2003). Also, Han et al. (2020), showed that addition of 4% soy protein isolate to soy milk and then fermentation with *Streptococcus thermophillus* caused an increase in GABA content by 1.5 fold higher than the sample without SPI addition (Han, Liao, Wu, Gong, & Bai, 2020). Karimian et al. (2020) reported that inoculation of proteolytic LAB and addition of SPI to the cheese whey resulted in increase in GABA content in the fermented whey. Proteolytic activity of starter cultures not

only increased the release of GABA precursors, but also reduce the fermentation time to reach to a desired pH (Karimian et al., 2020).



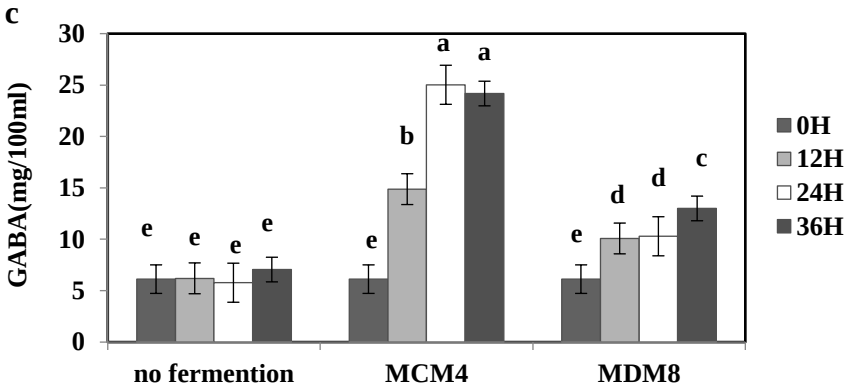
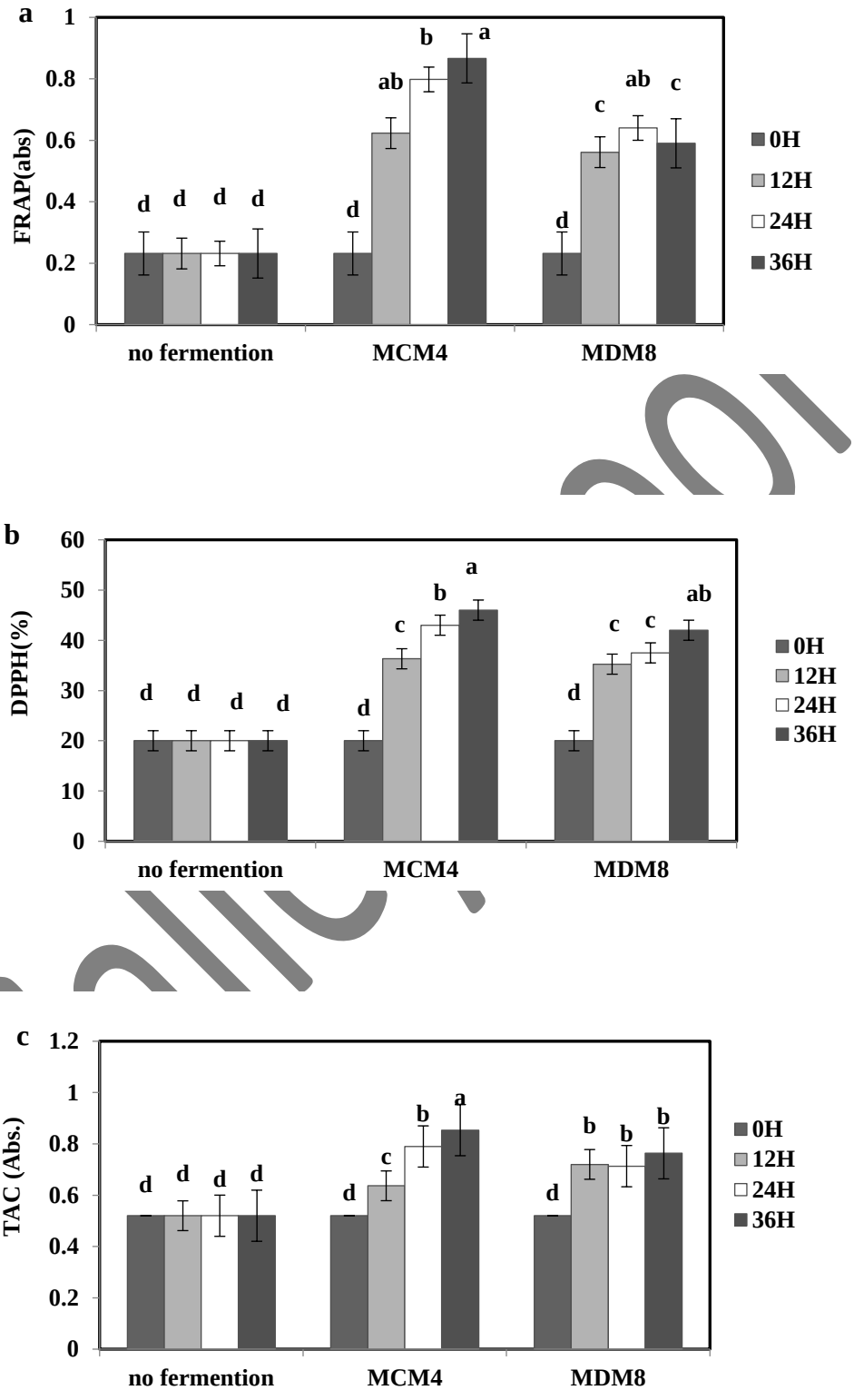


Figure 7- The content of GABA in soy whey fermented with *L. plantarum* MCM4 and *W. confusa* MDM8 (a) main effect of bacteria, (b) main effect of time and (c) interaction effect of bacteria and time on GABA content. Different letters on each column indicate significant differences between samples ($p<0.05$).

Effect of fermentation on antioxidant activity

Different antioxidant compounds may act against oxidizing agents through distinct mechanisms. Consequently, a single method cannot comprehensively evaluate the antioxidant capacity of complex matrixes (Xiao et al., 2015). Therefore, three antioxidant capacity assays with various approaches and mechanisms were employed to assess the antioxidant capacities of fermented soy whey, and the results are presented in Fig 8. Overall, significant differences were observed between the two bacterial species examined, and the ferric reducing power of the samples increased with fermentation time (Fig 8-a). According to the results shown in this figure, the highest ferric reducing power at 36 hours was observed in the *L. plantarum* MCM4 sample, while the lowest ferric reducing power was noted in the non-fermented sample.


a
FRAP(abs)
1
0.8
0.6
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d d d d
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0H
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DPPH(%)
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no fermention
MCM4
MDM8
0H
12H
24H
36H
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TAC (Abs.)
1.2
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0.4
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no fermention
MCM4
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0H
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24H
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Figure 8- Effect of fermentation on antioxidant activity in fermented soy whey: ferric reducing antioxidant power (FRAP) (A), DPPH radical scavenging activity (B), and total antioxidant activity (C).

Different letters on each column indicate significant differences between the samples ($p < 0.05$).

The results of the DPPH free radical scavenging assay are shown in Figure 8-b. As observed, the fermentation process significantly increased DPPH radical scavenging activity, influenced by both the bacterial species and the fermentation time. Extending the fermentation time up to 24 hours led to an increase in DPPH radical scavenging, while no significant change in scavenging activity was observed beyond 24 hours. Additionally, *L. plantarum* MCM4 demonstrated better performance compared to *W. confusa*.

Regarding the total antioxidant capacity, both the bacterial species and the fermentation time had a significant effect on the dependent variable ($p < 0.05$). Additionally, *L. plantarum* MCM4 exhibited better performance than *W. confusa* after 36 hours of fermentation (Fig. 8-c).

Overall, it was observed that fermentation positively affects total phenolic content and antioxidant activity. However, the degree of impact depends on the species of microorganisms used. It has been shown that lactic acid bacteria increase the aglycone isoflavone content in fermented soy whey due to high beta-glucosidase activity, and the released aglycone form can act as an antioxidant (Hur, Lee, Kim, Choi, & Kim, 2014). In the fermentation of soy whey by various lactic acid bacteria species, including *L. plantarum* and *S. thermophilus*, it has

been reported that extending the fermentation time from 24 hours to 48 hours leads to increased reducing power and DPPH free radical scavenging activity in the fermented sample (Monajjemi, Aminin, Ilkhani, & Mollaamin, 2012). Xiao et al. (2015) reported that fermenting soy whey with *L. plantarum* results in an increased ferric reducing power. These researchers attributed the increased reducing power in the fermented sample compared to the control to the release of iron-chelating compounds and the production of phenolic compounds during fermentation (Xiao et al., 2015). Additionally, in another study, soy samples fermented using two different proteolytic *Bacillus subtilis* isolates, *B. subtilis* MTCC5480 and *B. subtilis* MTCC1747, showed increased DPPH radical scavenging activity and reducing power compared to non-fermented soy. This was attributed to the high level of protein hydrolysis, increased TPC, and free amino acid content during fermentation (Sanjukta, Rai, Muhammed, Jeyaram, & Talukdar, 2015).

Conclusion

This study was done with the aim to enhance the amounts of bioactive compounds in SW using LAB. At first, the growth ability of eight LAB isolated from sourdough, raw milk, cabbage pickle and fermented olive in SW. From all tested isolates, *L. plantarum* MCM4 and *W. confuse* MDM8 displayed better performance in terms of acidifying capacity, and enhancing TPC and FAA content. The mentioned strains grew well in SW and when inoculated to SW caused increase in FAA content, TPC, GABA content and antioxidant activity. Regarding to the potential of the mentioned LAB strains, and their growth ability in SW, they can be used for the development of soy-based fermented products. For the better understanding of the mechanism behind bioactivity of SW, and optimization of fermentation conditions, it would be useful to identify phenolic compounds and isoflavons released during fermentation.

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افزایش فعالیت آنتی اکسیدانی و محتوای ترکیبات زیست فعال در آب پنیر سویا تخمیر شده با *Lactiplantibacillus plantarum* و *Weissella confuse*

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چکیده

آب پنیر سویا محصول جانبی فرایند تولید پنیر توفو و ایزوله پروتئین سویا است که دارای مقدار قابل توجهی ترکیبات مغذی مانند پروتئین، آمینواسید، اولیگوساکارید و ایزوفلاون می‌باشد. در این پژوهش، تخمیر آب‌پنیر سویا با استفاده از باکتری‌های اسید لاکتیک با هدف افزایش محتوای ترکیبات فنلی و گاما-آمینو بوتیریک اسید و فعالیت آنتی اکسیدانی محصول تخمیر شده صورت گرفت. برای این منظور، ابتدا ۸ سویه لاکتیکی مختلف غربال گری شدند و در مرحله بعد فعالیت موثرترین سویه‌ها و شمارش سلولی آن‌ها در طول تخمیر بررسی شد. نتایج نشان داد تمامی جدایه ها قادر به رشد در آب پنیر سویا بودند و افزایش زمان گرمخانه‌گذاری باعث کاهش معنی دار pH تمام نمونه ها از ۵/۷۵ به ۴/۵ شد. از بین ۸ جدایه LAB، *lactiplantibacillus plantarum* MCM4 و *Weissella confusa* MDM8 فعالیت بالاتری از نظر تولید اسید، افزایش محتوای TPC و فعالیت پروتئولیتیک نشان دادند. نمونه تخمیر شده توسط *L. plantarum* MCM4 بیشترین محتوای آمینو اسیدهای آزاد (۱/۷۳ میلی‌گرم در میلی‌لیتر) و نمونه تخمیر نشده با ۰/۹ میلی‌گرم در میلی‌لیتر کمترین مقدار را داشت. علاوه بر این، بیشترین میزان آمینو اسید آزاد پس از تخمیر ۳۶ و ۲۴ ساعت در نمونه تخمیر شده با *L. plantarum* MCM4 مشاهده شد. غلظت گابا از ۶/۱۵ میلی-گرم در ۱۰۰ میلی‌لیتر (تخمیر نشده) تا ۲۴/۱۷۵ میلی‌گرم در ۱۰۰ میلی-لیتر (SW تخمیر شده با *L. plantarum* MCM4) متغیر بود. همچنین همبستگی مثبتی بین شمارش سلولی و شدت پروتئولیز مشاهده شد. به طور کلی *L. plantarum* و *W. confusa* به خوبی در آب‌پنیر سویا رشد کردند و منجر به افزایش ترکیبات بالقوه زیست‌فعال در محصول نهایی شدند. بنابراین، تخمیر با *L. plantarum* MCM4 و *W. confusa* MDM8 می‌تواند به عنوان روشی برای ایجاد ارزش افزوده در آب‌پنیر سویا در نظر گرفته شود.

واژه های کلیدی: باکتری‌های لاکتیک اسید، زیست پالایش، گاما-آمینوبوتیریک اسید، تخمیر

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