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Enhancement of Antioxidant Activity and Bioactive Compounds in Soy Whey Fermented with *Lactiplantibacillus plantarum* and *Weissella confusa*

Sh. Atashgahi[1], A. Moayedi[2*], A. Sadeghi Mahoonak[3], H. Shahiri Tabarestani[4], A.R. Sadeghi[2]

1, 2, 3 and 4- M.Sc. Graduate, Associate Professor, Professor and Assistant Professor, Department of Food Science and Technology, Faculty of Food Science, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran, respectively.
(*- Corresponding Author Email: amoayedi@gau.ac.ir)

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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. Eight different LAB strains were selected and 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 led to significantly ($p<0.05$) decrease 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). 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 sample fermented 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, Fermentation, Gamma aminobutyric acid, Lactic acid bacteria

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 as a rich nutritional components 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

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is an expensive operation. Because of the lack of appropriate technology and enough economic motivation for SW recycling, 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 vital 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 elements (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 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) were 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 were kept as frozen cultures in the microbial bank.

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 method at 0, 12, 24 and 36 h of incubation 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):

Table 1- 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)

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

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 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 according to the method of 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 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 made up the volume to 125 ml. Aliqoute of 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 blank 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 examined 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.

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 samples, and there was significant difference among various LAB tested.

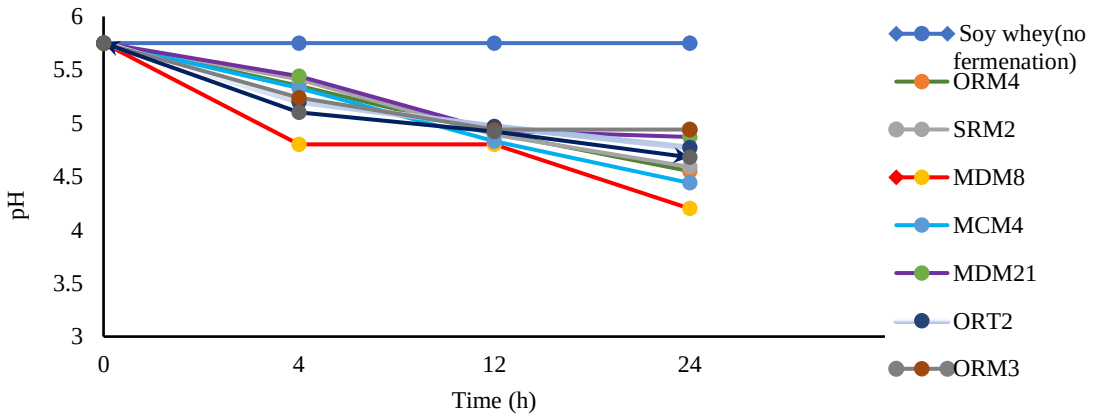

Fig. 1. pH levels in soy whey fermented by different LAB isolates

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).

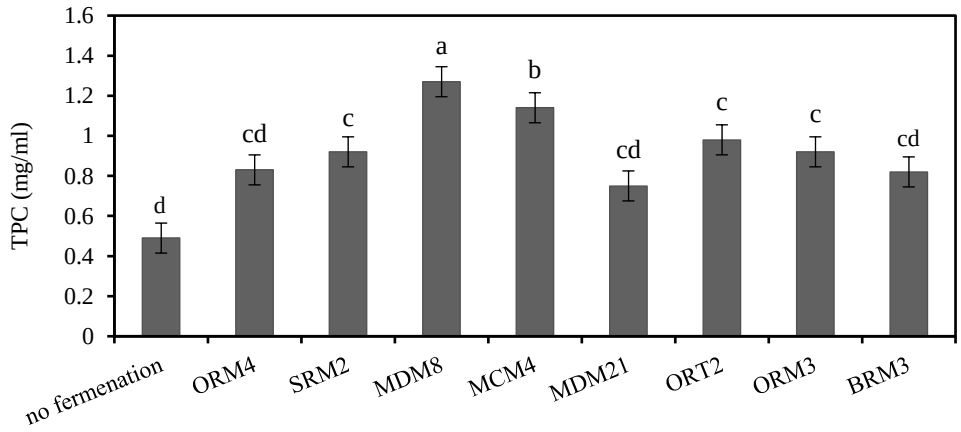

Fig. 2. Variations in the total phenolic content (TPC) of fermented soy whey (after 24 hours) by eight lactic acid bacteria isolates

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.

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 up 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 after 12 to 18 h of soy milk fermentation (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).

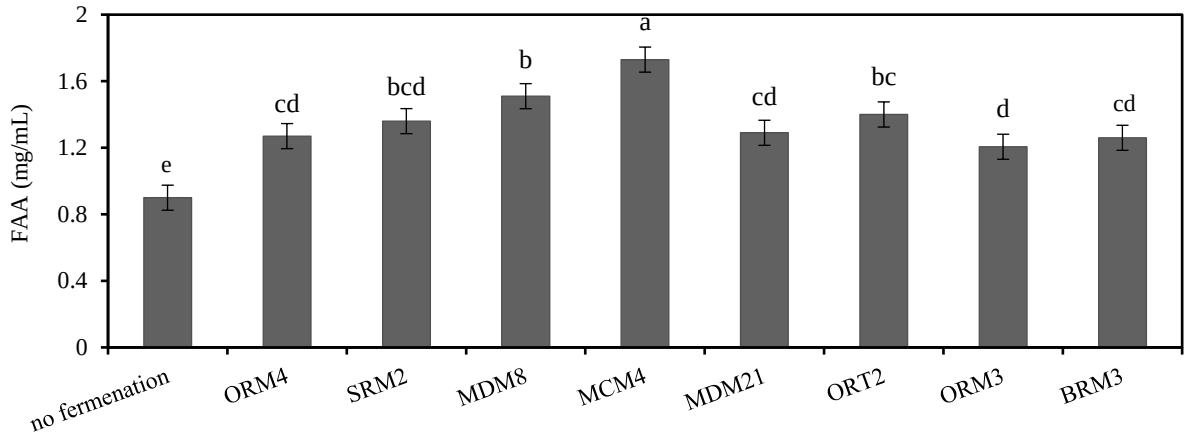

Fig. 3. The content of free amino group in soy whey fermented by 8 isolates of lactic acid bacteria during 24 hours of fermentation

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

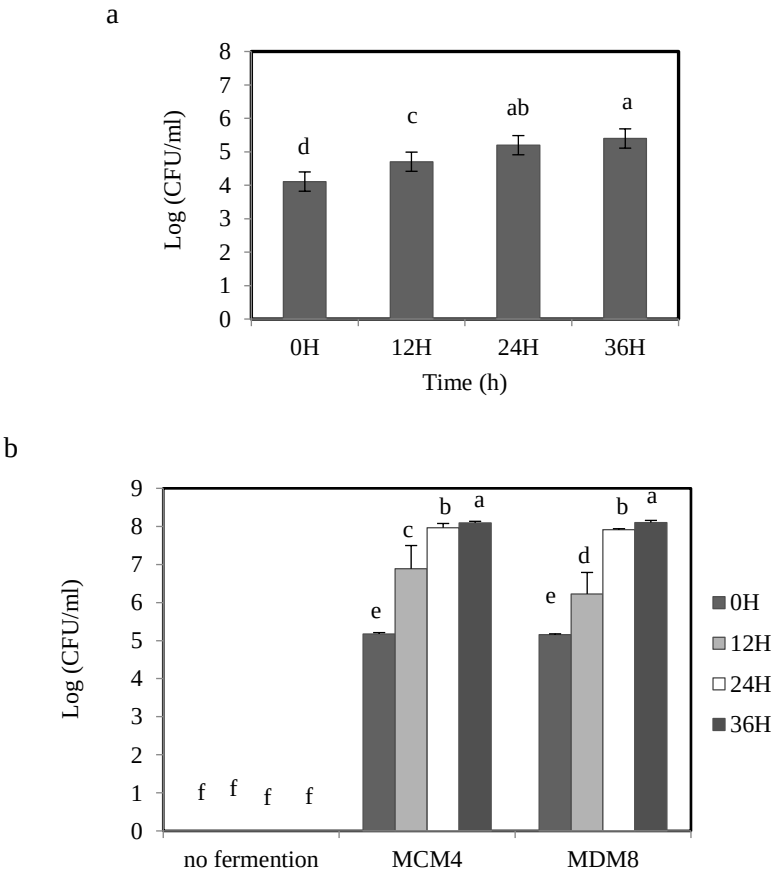

Fig. 4. Cell count in soy whey fermented by 2 isolates of *Lactiplantibacillus plantarum* MCM4 and *Weissella confusa* MDM8 (a) main effect of time and (b) interaction effect of isolate type and time on cell counts

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 bacteria (Lim, Foo, Loh, Mohamad, & Abdullah, 2019). In the current study, proteolytic activity of two LAB strains was investigated in SW during 36 h of 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).

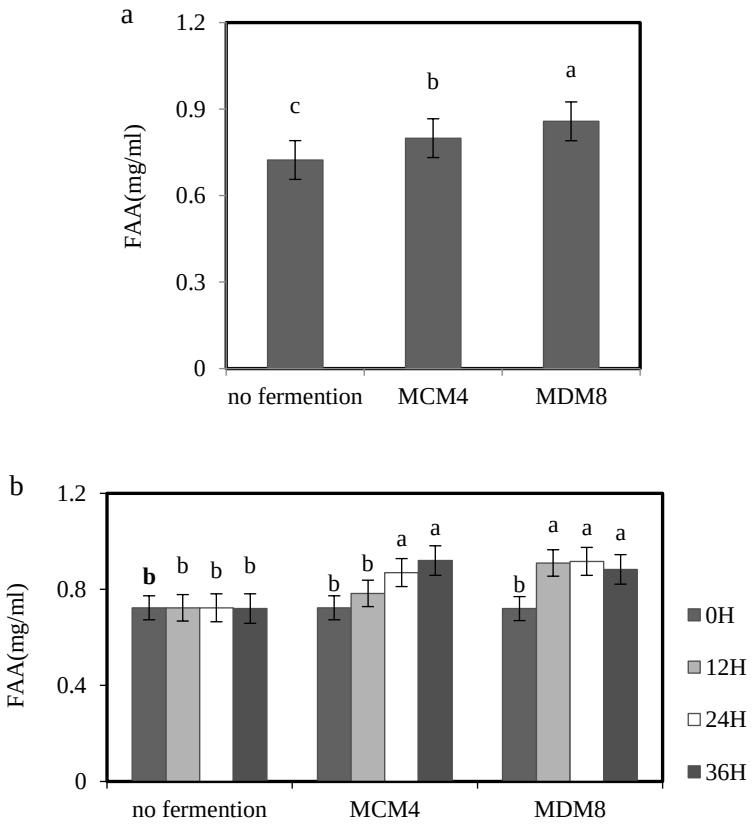

Fig. 5. Free amino acid (FAA) content in soy whey fermented with *L. plantarum* MCM4 and *W. confusa* MDM8. (a) The main effect of bacteria and (b) the interaction effect of bacteria and time on free amino group content
Different letters on each column indicate significant differences among the samples ($p<0.05$).

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 (Li *et al*., 2020).

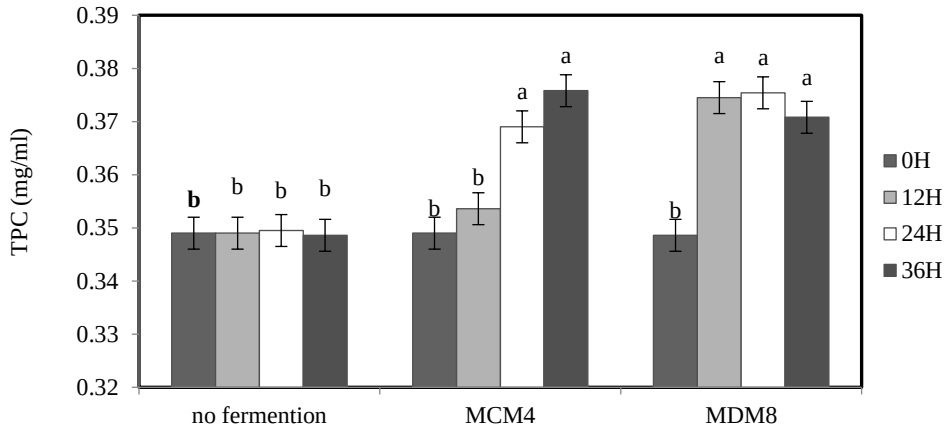

Fig. 6. The phenolic compound content in soy whey fermented with *L. plantarum* MCM4 and *W. confusa* MDM8 during fermentation

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 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 to produce GABA. 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).

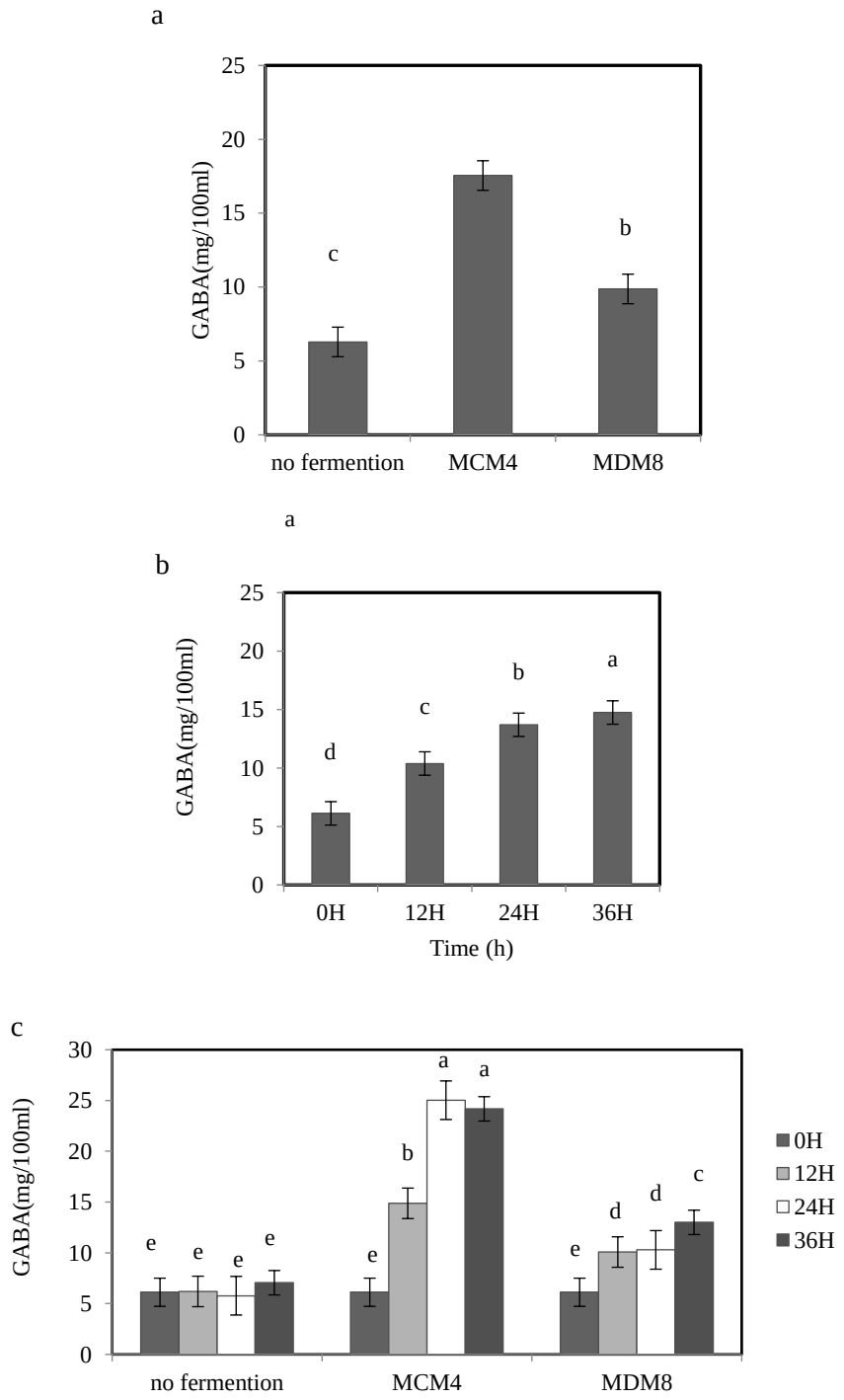

Fig. 7. The content of GABA in soy whey fermented with *L. plantarum* MCM4 and *W. confusa* MDM8 (a) main effect of bacteria, (b) main effect of time and (c) interaction effect of bacteria and time on GABA content
Different letters on each column indicate significant differences between samples ($p<0.05$).

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 matrices (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 *L. plantarum* MCM4 sample, while the lowest ferric reducing power was noted in the non-fermented sample.

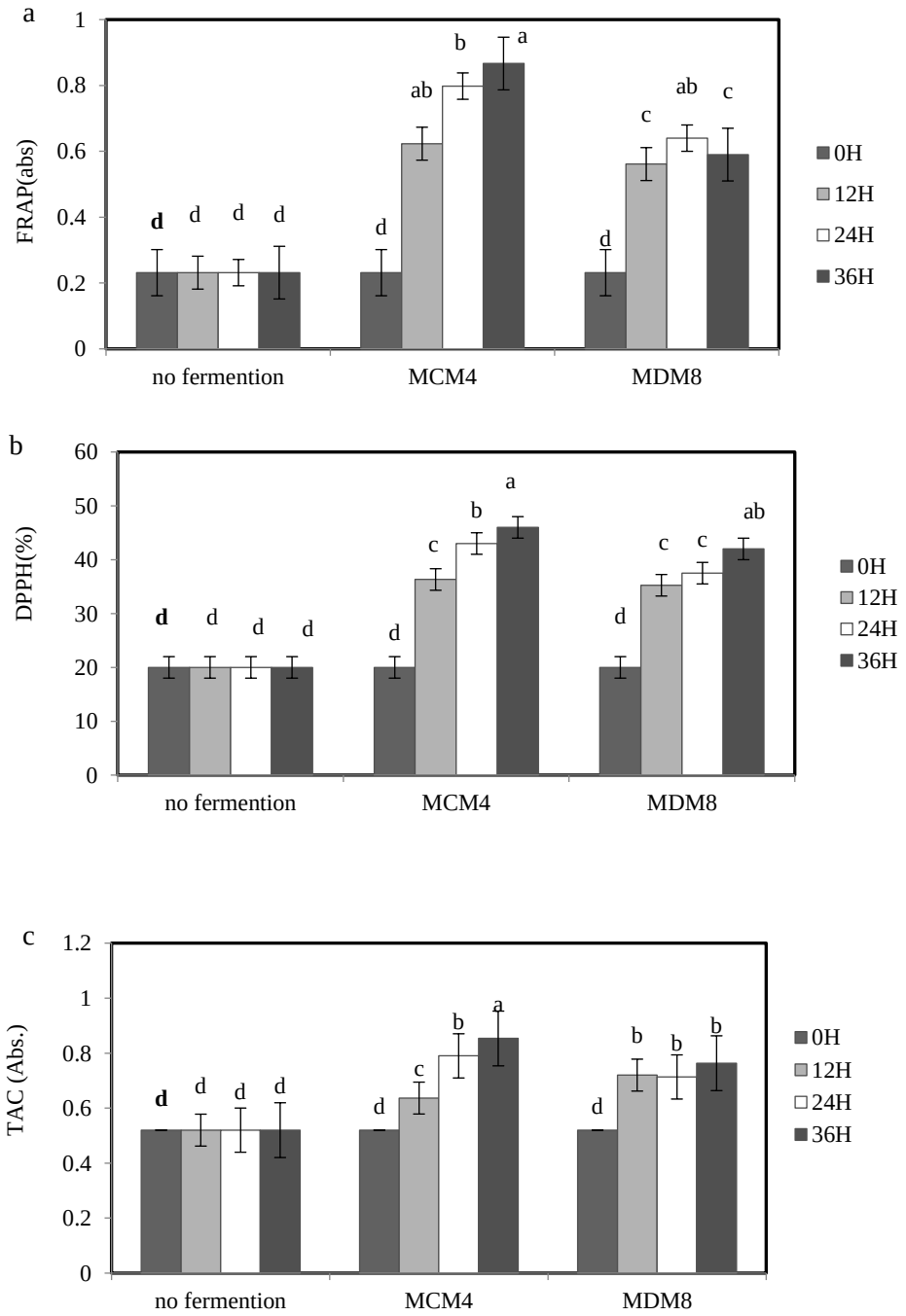

Fig. 8. Effect of fermentation on antioxidant activity in fermented soy whey: ferric reducing antioxidant power (FRAP) (A), DPPH radical scavenging activity (B), and total antioxidant activity (C)

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 Fig. 8-b. As viewed, 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 increased 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 aimed 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 was investigated. From all examined 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 an 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.

Author Contributions

S. Atashgahi: Funding acquisition, investigation, writing-original draft; **A. Moayedi:** Project administration, supervision, conceptualization, writing–review and editing; **A. Sadeghi Mahoonak:** Data curation, methodology; **H. Shahiri Tabarestani:** Formal analysis, software, writing–review and editing; **A.R. Sadeghi:** Data curation, validation.

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افزایش فعالیت آنتی‌اکسیدانی و محتوای ترکیبات زیست‌فعال در آب‌پنیر سویا تخمیر شده با *Lactiplantibacillus plantarum* و *Weissella confusa*

شادی آتشگاهی[۱]- علی مؤیدی[۲*]- علیرضا صادقی ماهونک[۳]- هدی شهیری طبرستانی[۴]- علیرضا صادقی[۲]

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چکیده

آب‌پنیر سویا محصول جانبی فرآیند 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 آنتی‌اکسیدانی محصول تخمیر شده صورت گرفت. برای این منظور، ابتدا ۸ سویه لاکتیکی مختلف غربال‌گری شدند و در مرحله بعد فعالیت مؤثرترین سویه‌ها و شمارش سلولی آن‌ها در طول تخمیر بررسی شد. نتایج نشان داد تمامی جدایه‌ها قادر به رشد در آب‌پنیر سویا بودند و افزایش زمان گرمخانه‌گذاری باعث کاهش معنی‌دار pH تمام نمونه‌ها 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. به‌طور 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 به‌عنوان روشی برای ایجاد ارزش افزوده در آب‌پنیر سویا در نظر گرفته شود.

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

۱، ۲، ۳ و ۴- به‌ترتیب دانش‌آموخته کارشناسی ارشد، دانشیار، استاد و استادیار گروه علوم و صنایع غذایی، دانشکده صنایع غذایی، دانشگاه علوم کشاورزی و منابع طبیعی گرگان، گرگان، ایران

(*- نویسنده مسئول: Email: amoayedi@gau.ac.ir)

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