



RESEARCH ARTICLE

REVISED Toxicology Study and MMP-1 and COL1A1 Expression of Malang Green Apple Juice Fermented by *Lactobacillus plantarum* DAD-13 and *Saccharomyces cerevisiae* Fermipan on Human Fibroblast: Potential Modality of Anti-aging

[version 2; peer review: 2 approved with reservations]

Mirna Theresia Eka Wardana ¹, Abrory Agus Cahya Pramana²,
Dyah Ayu Mira Oktarina ³

¹Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada, Yogyakarta, Special Region of Yogyakarta, Indonesia²Department of Biochemistry, Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada, Yogyakarta, Special Region of Yogyakarta, Indonesia³Department of Dermatology and Venereology, Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada, Yogyakarta, Special Region of Yogyakarta, Indonesia

v2 First published: 07 Nov 2025, 14:1226
<https://doi.org/10.12688/f1000research.166528.1>
 Latest published: 22 Dec 2025, 14:1226
<https://doi.org/10.12688/f1000research.166528.2>

Abstract

Background

Continuous exposure to sunlight and pollution without adequate protection can damage skin, making it prone to premature aging. One of the natural ingredients for anti-aging is antioxidant. Malang green apple (*Malus sylvestris* Mill) contains bioactive compounds that can act as antioxidants, but its use is still limited to only as food ingredient. Furthermore, some studies show the potency of fruit fermentation to increase these bioactive compounds. Thus, this study is a preliminary study to examine the anti-aging potency of fermented Malang green apples.

Methods

Human foreskin fibroblasts were treated with fermented Malang green apple juice extract (FAJ) by *Lactobacillus plantarum* DAD-13 and *Saccharomyces cerevisiae* Fermipan and its unfermented (AJ) for 24, 48, and 72 hours. Cell viability was measured using the MTT assay and collagen deposition was measured using the Sirius red staining method. The expression of matrix metalloproteinase 1 (MMP-1) and

Open Peer Review

Approval Status ? ?

	1	2
version 2		
(revision)		
22 Dec 2025	view	view
version 1		
07 Nov 2025		
1. Phaniendra Alugoju  , Palacký University, Šlechtitelů, Czech Republic		
2. Md Suzauddula Suzauddula  , Kansas State University, Manhattan, USA		
Any reports and responses or comments on the article can be found at the end of the article.		

collagen type 1 alpha 1 chain (COL1A1) was measured in fibroblasts after treatment with extracts.

Results

Cell viability and collagen deposition of fibroblasts tended to be higher after 48 h of extract exposure. Fibroblasts that received FAJ 62.5 µg/mL showed higher cell viability than negative control ($p < 0.05$) and FAJ 31.25 µg/mL showed higher collagen deposition than negative control ($p < 0.05$) at 48 hours of extract exposure. AJ and FAJ also decreased MMP-1 gene expression. They showed a significant decrease in MMP-1 levels ($p < 0.01$) compared to the untreated group, whereas they showed increased expression of COL1A1 at low concentrations. There was no significant difference in the gene expression between FAJ and AJ.

Conclusions

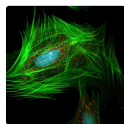
Our results indicated that Malang green apple juice extract affected fibroblast cell viability, collagen deposition, MMP-1, and COL1A1 gene expression. Exposure of lower concentrations of FAJ to skin fibroblasts for 48 hours showed better results.

Keywords

Malang green apple juice, fermentation, anti-aging, MMP-1, COL1A1, *Lactobacillus plantarum*, *Saccharomyces cerevisiae*



This article is included in the **Plant Science** gateway.



This article is included in the **Advances in Fibroblast Research** collection.

Corresponding author: Dyah Ayu Mira Oktarina (d.oktarina@ugm.ac.id)

Author roles: Wardana MTE: Data Curation, Formal Analysis, Investigation, Writing – Original Draft Preparation; Pramana AAC: Methodology, Writing – Review & Editing; Oktarina DAM: Conceptualization, Funding Acquisition, Resources, Supervision

Competing interests: No competing interests were disclosed.

Grant information: This study was funded by the Faculty of Medicine, Public Health, and Nursing, Gadjah Mada University under the program “Hibah Dana Masyarakat (DAMAS)”.

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Copyright: © 2025 Wardana MTE *et al.* This is an open access article distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

How to cite this article: Wardana MTE, Pramana AAC and Oktarina DAM. **Toxicology Study and MMP-1 and COL1A1 Expression of Malang Green Apple Juice Fermented by *Lactobacillus plantarum* DAD-13 and *Saccharomyces cerevisiae* Ferment on Human Fibroblast: Potential Modality of Anti-aging [version 2; peer review: 2 approved with reservations]** F1000Research 2025, 14:1226 <https://doi.org/10.12688/f1000research.166528.2>

First published: 07 Nov 2025, 14:1226 <https://doi.org/10.12688/f1000research.166528.1>

REVISED Amendments from Version 1

For version 1, we decided to change the authorship order between the second author and the third author based on their roles in this research. We also have made some changes regarding the methodology to explain the component of media culture and give more details about the volume of reagents used in this research.

Any further responses from the reviewers can be found at the end of the article

Introduction

Aging is a naturally occurring biological process that differs among individuals.¹ Given its feature as the outermost part of the human body, signs of aging in the skin are more noticeable by the human eye than in other organs.^{1,2} Skin aging can be divided into intrinsic and extrinsic aging.^{3,4} Intrinsic aging is a physiological process characterized by a progressive decline in body functions.^{3,5} On the other hand, extrinsic aging is estimated to be the cause of 80% of all signs of aging, and is caused by a multitude of factors, including UV exposure, air pollution, and lifestyle factors such as diet and smoking.⁶

Ultraviolet irradiation causes damage to fibroblasts by reducing collagen synthesis, increasing the rate of its breakdown, promoting oxidative stress, and increasing the content of matrix metalloproteinases (MMP) that help break down collagen.⁷ Various types of MMPs are responsible for pathophysiological processes, not only limited to aging; however, MMP-1 degrades type I and III collagen, where collagen is responsible for the elasticity and strength of the skin.⁸

Ingredients with anti-aging effects can inhibit the aging process. The use of materials with antioxidants is one way to prevent aging by reducing and neutralizing reactive oxygen species (ROS).^{4,5,9,10} Antioxidants can be obtained from natural ingredients such as fruits and vegetables, including apples.¹¹ Green apples contain catechins, vitamin C, quercetin, tocopherols, and carotenoids.^{12,13} The Malang green apple (*Malus sylvestris* mill) contains a high amount of quercetin, a natural antioxidant.¹⁴ Thus, Malang green apple has the potential to be developed as an anti-aging ingredient. Unfortunately, Malang green apple is limited to food processing. On the other hand, fermentation can increase a material's bioactive compounds. Fermentation by lactic acid bacteria in fruits has been shown to increase their antioxidant activity.¹⁵ Thus, in this study, we investigated the effect of the fermentation process in Malang green apple juice, especially the green apple variety from Malang, Indonesia on cell viability and collagen deposition in skin fibroblasts. We also assessed MMP-1 expression in treated fibroblast cells. We hope that this research can add more benefit value to Malang green apple as a potential anti-aging ingredient.

Methods

Study design

This was an *in vitro* experimental study. This study was conducted by the Department of Dermatology and Venereology in collaboration with the Department of Biochemistry, Faculty of Medicine, Public Health, and Nursing, Gadjah Mada University.

Study procedure

1. Green apple juice making and pasteurization

Malang green apple were obtained from a traditional market in Malang, Indonesia. Green apple juice 70% (w:v) was prepared by adding 10 mL of 0.05 M citric acid and 10 ml of 100 mg/mL ascorbic acid to green apple slices that had previously been sprayed with 0.05 M citric acid. The addition of an acidifying agent can prevent enzymatic browning by reducing pH.¹³ It was crushed with a blender, filtered, and pasteurized at 105 °C in an autoclave for 1 minute.

2. Green apple juice fermentation

Pasteurized Malang green apple juice was fermented with 1.8% (v:v) *Lactobacillus plantarum* DAD-13 + 1.8% (v:v) *Saccharomyces cerevisiae* Fermipan for 1 day at 37 °C.

3. Green apple juice extraction

Fermented and non-fermented Malang green apple juice is added with ethyl acetate at a ratio of 1:1 (v:v) and macerated for 1 day. The ethyl acetate layer was extracted again using ethyl acetate. The filtrate was evaporated

on evaporation porcelain and then transferred to a glass vial (dried in an oven at 45 °C) to obtain fermented and non-fermented Malang green apple juice extracts.

4. Cell culture

Fibroblast cell lines were obtained from human foreskin. The growth process of fibroblast cell lines was conducted in the Laboratory of Dermatology Venerology, Department of Dermatology and Venereology, Faculty of Medicine, Public Health, and Nursing, Gadjah Mada University. Fibroblast cell lines were grown in Dulbecco's modification of Eagle's medium (DMEM, Gibco®, USA) with 10 mL of fetal bovine serum (FBS), 1 mL of 1% penicillin-streptomycin (Gibco®, USA), and 1 mL of amphotericin B. Cells were incubated in an incubator at 37 °C and 5% CO₂.

5. Cell viability with MTT assay

To conduct a cytotoxicity test on the fibroblast cell lines, the medium was aspirated and the cells were washed with phosphate-buffered saline (PBS) twice. The cells were then separated by trypsin (Gibco®, USA). Trypsin was diluted in DMEM after 4 minutes, and the detached cells were centrifuged for 10 minutes at 2000 rpm. The cells were counted and plated the cells in 96-well plates (100 µL solution per well contained 10.000 cells/well). The fibroblast cell lines were incubated for 24 hours (37 °C, 5% CO₂). The next day, the cells were treated with fermented green apple juice extract and unfermented green apple extract at various concentrations ranging from 15.826 µg/mL to 1000 µg/mL. The 96-well plates were then incubated overnight. After incubation, the medium in each well was aspirated, and 100 µL of MTT was added to create formazan.¹⁶ After 4 hours of incubation and formation of formazan, a stopper of 100 L SDS 10% in 0.1 N HCl was added and incubated overnight in the dark. The intensity of the purple color was measured using an ELISA reader at a wavelength of 595 nm.

6. Collagen deposition

Collagen deposition in fibroblasts was determined according to the method proposed by Benitez et al.¹⁷ with some modifications. Fibroblasts (10.000 cells/well) treated with the extracts were incubated for 24, 48, or 72 hours. After incubation, the medium was aspirated, and the cells were washed twice with PBS. Bouin's solution (100 µL) was added to each well and allowed to stand for 1 hour in the dark. Bouin's solution was rinsed with deionized water until the yellow color disappeared and was left overnight. The wells were filled with Sirius red stain (100 µL per well), and the 96-well plate was shaken for 1 hour. Give 0.01 N HCl, followed by 0.5 N HCl, and then shake the well for 30 minutes. The absorbance was read using an ELISA reader at 550 nm.

7. MMP-1 and COL1A1 quantification

Ribonucleic acid (RNA) was isolated from fibroblast cells using PRImeZOL™ reagent (RNA isolation reagent) according to the manufacturer's instructions (Canvax, America). The cells were homogenized with the reagent. The supernatant from the sample was separated by adding 0.2 mL of chloroform per 1 mL of the reagent. After that, it was centrifuged for 15 minutes at 12000 rpm. The aqueous layer, containing RNA, was transferred to a different tube and precipitated with cold 70% isopropyl alcohol, followed by incubation at room temperature. Then, the sample was centrifuged at 12000 rpm for 10 minutes at 4°C to obtain RNA pellet. Pellet was washed with 75% ethanol and then vortexed and centrifuged at 7500 rpm for 5 minutes at 4 °C. The RNA pellet was dissolved with nuclease-free water (NFW) and incubated at 60°C for 5 minutes. Isolated RNA was stored at -20 °C. The isolated RNA was then subjected to complementary deoxyribonucleic acid (cDNA) preparation process according to the manufacturer's instructions (SMOBio). Isolated RNA was mixed with 1µL of oligo (dT), and 10µL Diethyl pyrocarbonate (DEPC)-treated H₂O, spun down, and incubated at 70 °C for 5 minutes, mixed again with a cDNA synthesis mixture consists of 4µL 5-RTX buffer, 5µL DEPC treated H₂O, and 1µL RT-ase, and then incubated to make cDNA. The cDNA samples were diluted from 100ng/mL to 50ng/mL, 20µL of the sample was taken and added into 20µL of NFW, mixed by up and down method. The polymerase chain reaction (PCR) master mix was prepared by adding 10µL of the master mix, 0.8µL of the forward primer, 0.8µL of the reverse primer, 4.4 µL of NFW. Primers used were obtained from Integrated DNA Technologies (IDT), and the primer sequences are listed in Table 1. The 16µL of the PCR mix was mixed with 4µL of the sample. The results of the samples were read using a Bio-Rad thermocycler.

Table 1. Primer sequences for Beta Actin (ACTB) and MMP-1 gene.

Gene	Primer	Sequence
ACTB	Forward	5'TTCTCTGACCTGAGTCTCCTT3'
	Reverse	5'ACACCCACAACACTGTCTTAG3'
MMP-1	Forward	5'TTCCGCCTGTCTCAAGATGATAT3'
	Reverse	5'AAAGGACAAAGCAGGATCACAGTT3'

Data analysis

Cell viability and collagen deposition data were analyzed using two-way analysis of variance (ANOVA) and Dunnett's test for post hoc analysis. MMP-1 gene expression was analyzed using one-way ANOVA. Data analysis was done using SPSS while GraphPad Prism 9.3.0 were utilized to make a graph. For all tests, statistical significance was defined as $p < 0.05$.

Results

Cell viability with MTT assay

The effect of FAJ on fibroblast cell line viability was compared with that of the fibroblast cell line that received AJ after cells were exposed to the extract for 24, 48, and 72 hours (Figure 1). The analysis of the fibroblast cell line showed

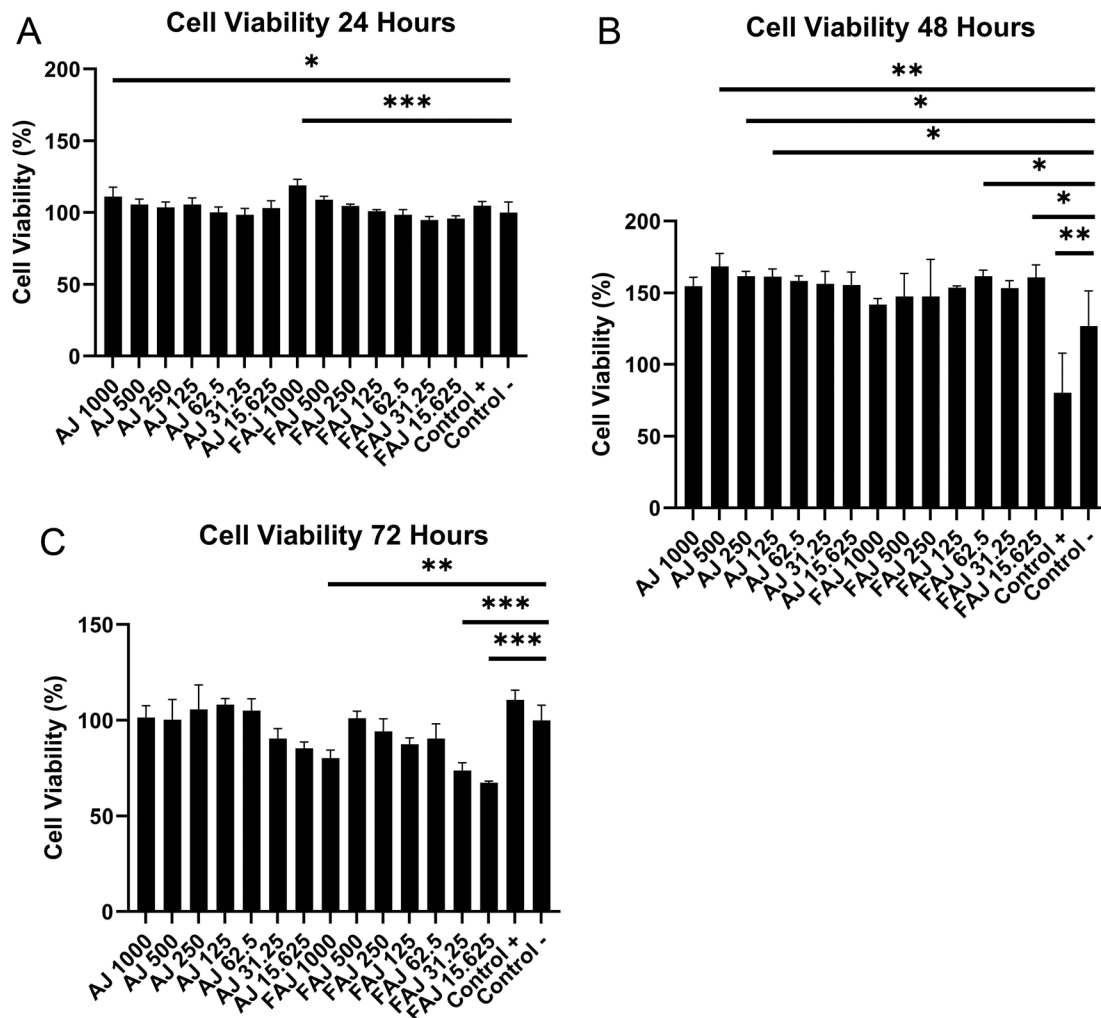


Figure 1. Effect of AJ and FAJ extract on fibroblast cell viability. Cell line received various concentrations of AJ and FAJ extracts for (A) 24 hours; (B) 48 hours; (C) 72 hours determined by MTT assay. The error bars depict mean values $\pm$ SD from triplicate experiments. The significance of difference was set to $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$ compared with non-treated fibroblasts cell line.

a significant effect between different types of extracts (FAJ and AJ) and cell viability at 48 and 72 hours of extract exposure, while the variation of extract concentration from 15.625 $\mu\text{g/mL}$ to 1000 $\mu\text{g/mL}$ had a significant effect on cell viability after 24 and 72 hours of extract exposure ($p < 0.05$). The viability of fibroblasts receiving FAJ was higher than that of fibroblasts receiving AJ on 24-hour extract exposure, except at the concentration of 62.5 $\mu\text{g/mL}$, but it was not statistically significant. In the 24-hour extract exposure group, fibroblasts receiving FAJ and AJ 1000 $\mu\text{g/mL}$ showed higher cell viability than the negative control. This pattern changed on 48-hour extract exposure. Fibroblasts receiving AJ at 125-500 $\mu\text{g/mL}$ concentration and FAJ at 15.625 and 62.5 $\mu\text{g/mL}$ concentration showed higher cell viability than the negative control ($p < 0.05$). The cell viability in FAJ was higher than that in AJ at 15.625 and 62.5 $\mu\text{g/mL}$. After receiving its peak at 48-hour extract exposure, cell viability decreased at 72-hour extract exposure. Cell viability in the fibroblast group of FAJ at the concentration of 15.625 – 31.25 $\mu\text{g/mL}$ and 1000 $\mu\text{g/mL}$ (72 hours) was lower than negative control ($p < 0.05$).

Collagen deposition

The effect of FAJ on fibroblast collagen deposition was compared with that of the fibroblast cell line that received AJ after cells were exposed to the extract for 24, 48, and 72 hours. Overall, the collagen deposition ratio (Figure 2) showed the same pattern as cell viability, which increased up to 48 hours and decreased after 72 hours of extract exposure, except in the fibroblasts group that received AJ 250-1000 $\mu\text{g/mL}$. The analysis carried out on the data showed that the duration of extract exposure (24, 48, and 72 hours) and variation of extract from 15.625 to 1000 $\mu\text{g/mL}$ had a significant effect on the collagen deposition ratio, while different types of extracts had a significant effect on the collagen deposition ratio after 24 and 72 hours extract exposure. The collagen deposition ratio in fibroblasts receiving FAJ at a concentration of 31.25 $\mu\text{g/mL}$ and AJ at 62.5 $\mu\text{g/mL}$ for 24 and 48 hours was higher than that in the negative control ($p < 0.05$).

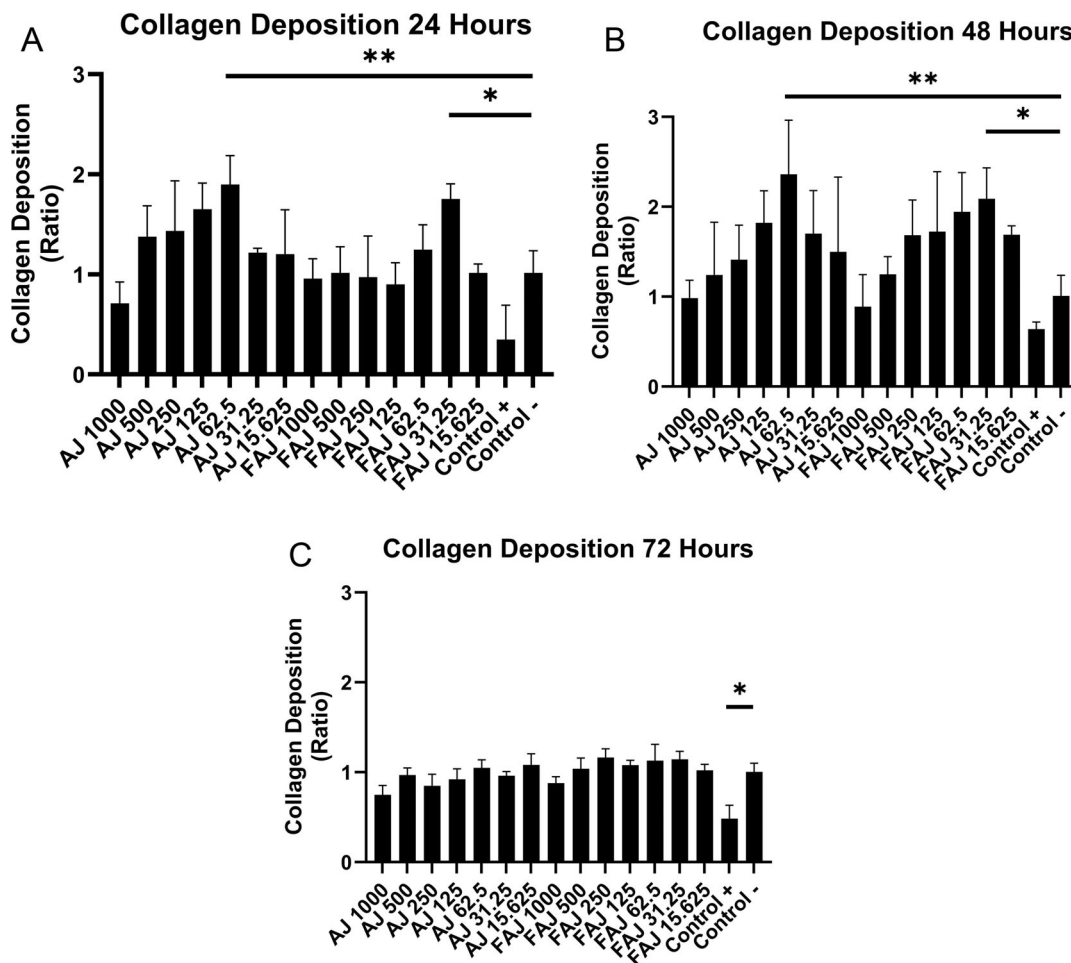


Figure 2. Effect of AJ and FAJ extract on collagen deposition in fibroblasts. Cell line received various concentrations of AJ and FAJ extracts for (A) 24 hours; (B) 48 hours; (C) 72 hours. The error bars depict mean values $\pm$ SD from triplicate experiments. The significance was set to $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$, compared with non-treated fibroblasts cell line.

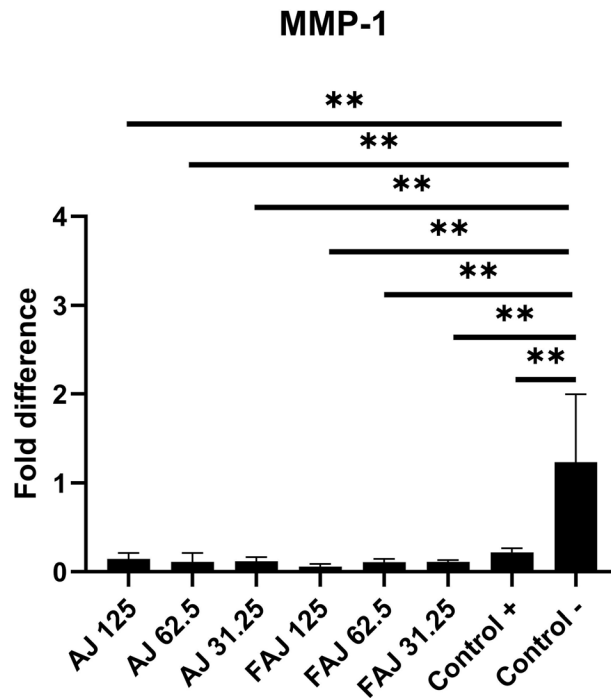


Figure 3. Expression of MMP-1 on fibroblasts treated with AJ and FAJ extract. The significance was set to $*p<0.05$, $**p<0.01$, and $***p<0.001$, compared with control.

MMP-1 and COL1A1 levels

MMP-1 gene expression (Figure 3) in fibroblasts treated with FAJ and AJ at concentrations of 31.25, 62.5, and 125 $\mu\text{g/mL}$ for 48 hours showed significant downregulation relative to that in the control ($p < 0.05$). MMP-1 levels in the FAJ group at concentrations of 31.25, 62.5, and 125 $\mu\text{g/mL}$ decreased by 89.02%, 89.63%, and 94.6%, respectively, compared to the control group, whereas in the AJ group, it decreased by 88.62%, 91.2%, and 86.6%, respectively, compared to the levels of MMP-1 in fibroblasts not treated with any extract. There were no significant differences in MMP-1 levels between AJ and FAJ. COL1A1 expression (Figure 4) also exhibited a dose-dependent pattern, with higher

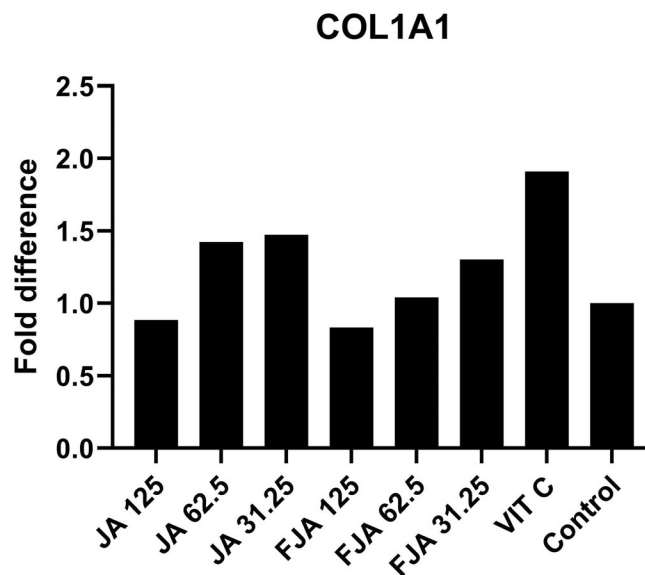


Figure 4. Expression of COL1A1 on fibroblasts treated with AJ and FAJ extract. The significance was set to $*p<0.05$, $**p<0.01$, and $***p<0.001$, compared with control.

concentrations of AJ and FAJ showing lower COL1A1 expression. COL1A1 levels in the FAJ group at concentrations of 31.25 and 62.5 µg/mL increased by 30% and 4%, respectively, compared to the control, whereas it decreased by 17% at a concentration of 125 µg/mL. In the AJ group, COL1A1 levels at concentrations of 31.25 and 62.5 µg/mL increased by 47% and 42%, respectively, compared to the control group, whereas it decreased by 12% at a concentration of 125 µg/mL. The changes in COL1A1 levels in both the AJ and FAJ groups were not statistically significant ($p > 0.05$).

Discussion

Our study conducted a toxicity study on fibroblasts receiving extracts from fermented and unfermented Malang green apple juice. We also conduct a study to know the expression of MMP-1 and COL1A1. In our study, exposure of human skin fibroblast to FAJ and AJ affected cell viability and collagen deposition. Fibroblasts that received FAJ and AJ showed an increase in cell viability along with increasing extract concentration at 24 hours exposure to FAJ 1000 µg/mL, which showed the highest increase compared to the negative control. However, this pattern changed after 48 hours of exposure. Fibroblasts that received low concentration of FAJ showed higher cell viability than those that received high concentration of FAJ, with a decrease starting at 62.5 µg/mL. In the AJ group, the pattern after 48 hours of exposure remained the same with 24 hours exposure group. Fibroblasts that received a low concentration of FAJ also showed higher collagen proliferation than those that received high concentrations.

The results of cell viability and collagen deposition were also reflected by MMP-1 and COL1A1 expression. Low concentrations of FAJ and AJ increased COL1A1 expression compared to untreated cells one and decreased COL1A1 expression at 125 µg/mL, although the change was not statistically significant. FAJ and AJ also decreased MMP-1 expression in a dose-dependent pattern, indicating that this extract can inhibit one of the genes that control collagen degradation. However, the decrease in MMP-1 levels at 125 µg/mL, which is too strong, and the decrease in COL1A1 levels at the same concentration might indicate a decrease in living fibroblasts.

Fibroblasts dominate the dermis.¹⁸ These cells play important roles in the formation and repair of skin components by producing collagen and extracellular matrix.¹⁹ Fibroblasts are skin cell types that have been widely used in vitro research to determine the effect of exposure to certain extracts exposure before being tested on skin tissue.²⁰ The fermentation process carried out on green apple juice could increase the bioactive compounds contained in apple juice, especially compounds that with antioxidant activity. Antioxidants play a role in neutralizing the effects of reactive oxygen species (ROS) such that they do not cause damage and aging in cells.²¹ Several previous studies have demonstrated the effect of fermentation on increasing antioxidant activity by increasing total phenol, anthocyanin, and flavonoid content, especially myricetin and quercetin^{22,23} as well as increasing the biological activity of polyphenols.²⁴ Quercetin present in green apples has antioxidant activity that increases the lifetime and viability of fibroblasts.²⁵ Flavonoids and polyphenols contained in apples are also predicted to protect cells from oxidative stress and increase cell viability.²⁶ In addition, polysaccharides produced by lactobacilli can increase cell proliferation because of their effect on fibroblast growth factors.²⁷

The higher collagen deposition ratio in the extract group than in the negative control was in accordance with previous studies. Fermentation of fruit and vegetable drinks using *Saccharomyces cerevisiae* and *Streptococcus thermophilus* TCI125 could increase collagen levels.²⁸ The fermented extract can increase collagen levels, especially collagen type I, by inhibiting mRNA expression and collagenase production.²⁹ Phenolic compounds found in apples can inhibit collagen degradation by inhibiting matrix metalloproteinase 1 (MMP-1) and these compounds also prevent cell damage from free radicals.³⁰

Collagen, especially type I collagen, is the most abundant protein in skin connective tissue and is responsible for maintaining skin structure.³¹ Reduction in fibroblasts and collagen fragmentation due to chronological age or ultraviolet radiation can disturb skin elasticity and cause damage to the skin.^{32,33} Increased production of MMP-1 caused by exposure to intrinsic and extrinsic factors is strongly related to collagen fragmentation, therefore, a decrease in its production may correlate with collagen increase and later have an anti-aging effect.^{32,34} Several previous studies have shown that fermented extract can decrease the expression of MMP-1.^{35,36} In our study, both Green Malang apple juice extracts significantly inhibited the expression of MMP-1. This indicates that FAJ and AJ exert anti-aging properties by decreasing one of the main causes of collagen degradation.

Our study showed better results with low FAJ concentrations. This may have been caused by some factors. First, the antioxidant activity of the FAJ extract reached its maximum activity, thus reducing the antioxidant efficacy.³⁷ The decrease in antioxidant activity due to the fermentation process has been shown by a decrease in total phenol content in strawberry fermentation due to anthocyanin condensation.³⁸ Red cabbage fermentation using *Lactobacillus acidophilus* and *Lactobacillus plantarum* caused a decrease in anthocyanin content and total phenolic content after 7 days of

fermentation.³⁹ Two, the fermentation process of green apple juice increased the acidity of the extract, which can negatively impact the fibroblasts. *Saccharomyces cerevisiae* and *Lactobacillus plantarum* can further decrease the pH of the juice more than a single fermentation.⁴⁰ Fibroblasts in an acidic environment (pH 5.5) showed lower proliferation activity than those in a neutral or base environment.⁴¹ Three, the formation of pro-oxidant activity. A previous study showed that papierowka apple peel extract had a high polyphenol content and high pro-oxidant activity because phenolic components could also initiate the formation of ROS.²³ Overall, changes in the phenolic components that play a role in antioxidant components and activity are influenced by the probiotic strain, fermentation temperature, fermentation substrate, and fermentation period.^{42–44}

This study provides preliminary information on the effects of the fermentation process of local green apple juice from Malang, Indonesia on normal skin fibroblasts. Malang green apple juice (fermented and unfermented) affected cell viability, collagen deposition, and MMP-1 and COL1A1 levels in the skin fibroblasts. Exposure of skin fibroblast to fermented Malang green apple juice at low concentrations for 48 hours gave better results than exposure to high concentrations, given that the high concentration might undergo increased pH level, decreased antioxidant activity over time, or pro-oxidant activity.

Our study was limited by the absence of high-performance liquid chromatography (HPLC) data to analyze the type and amount of compounds in FAJ, the pH value before and after fermentation, antioxidant activity, and the effect of different probiotic strains, temperature, and fermentation time on cell viability and collagen deposition in normal fibroblasts.

Ethical considerations

Ethical approval for this study was approved by the Ethics Committee of the Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada, with reference number KE/FK/0143/EC/2022 on February 9th 2022.

Data availability

Underlying data

Figshare: Toxicology Study and MMP-1 and COL1A1 Expression of Malang Green Apple Juice Fermented by *Lactobacillus plantarum* DAD-13 and *Saccharomyces cerevisiae* Fermipan on Human Fibroblast: Potential Modality of Anti-aging. <https://doi.org/10.6084/m9.figshare.28233320.v3>.⁴⁵

This project contains the following underlying data:

- Data file 1: Datasets

Data are available under the terms of the [Creative Commons Attribution 4.0 International license](#) (CC-BY 4.0).

Extended data

No extended data are associated with this article.

Reporting guidelines

Figshare: Dataset for 'Toxicology Study and MMP-1 and COL1A1 Expression of Malang Green Apple Juice Fermented by *Lactobacillus plantarum* DAD-13 and *Saccharomyces cerevisiae* Fermipan on Human Fibroblast: Potential Modality of Anti-aging', <https://doi.org/10.6084/m9.figshare.28233320.v3>.⁴⁵

Acknowledgements

The authors extend their gratitude to the Department of Dermatology and Venereology and the Department of Biochemistry at the Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada, Yogyakarta for making this study a success.

References

1. Jang JD, Kim M, Nam GH, et al.: **Antiaging Activity of Peptide Identified from Fermented *Trapa Japonica* Fruit Extract in Human Dermal Fibroblasts.** *Evid-Based Complement Altern. Med. ECAM*. 2020; **2020**: 5895029. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
2. Garza L: **Developmental Biology of the Skin.** In: *Fitzpatrick's Dermatology*. 2019 [cited 2022 Jan 2]; 9e. [Reference Source](#)
3. de Araújo R, Lôbo M, Trindade K, et al.: **Fibroblast Growth Factors: A Controlling Mechanism of Skin Aging.** *Skin Pharmacol. Physiol*.

- 2019; **32**(5): 275–282.
[PubMed Abstract](#) | [Publisher Full Text](#)
4. Zhang S, Duan E: **Fighting against Skin Aging: The Way from Bench to Bedside.** *Cell Transplant.* 2018 May; **27**(5): 729–738.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 5. Bosch R, Philips N, Suárez-Pérez JA, et al.: **Mechanisms of Photoaging and Cutaneous Photocarcinogenesis, and Photoprotective Strategies with Phytochemicals.** *Antioxid. Basel Switz.* 2015 Mar 26; **4**(2): 248–268.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 6. Rodan K, Fields K, Majewski G, et al.: **Skincare Bootcamp: The Evolving Role of Skincare.** *Plast. Reconstr. Surg. Glob. Open.* 2016 Dec; **4**(12 Suppl Anatomy and Safety in Cosmetic Medicine: Cosmetic Bootcamp): e1152.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 7. Yamaba H, Haba M, Kunita M, et al.: **Morphological change of skin fibroblasts induced by UV Irradiation is involved in photoaging.** *Exp. Dermatol.* 2016 Aug; **25**(Suppl 3): 45–51.
[PubMed Abstract](#) | [Publisher Full Text](#)
 8. Bonté F, Girard D, Archambault JC, et al.: **Skin Changes During Ageing.** *Subcell. Biochem.* 2019; **91**: 249–280.
[Publisher Full Text](#)
 9. Petruk G, Del Giudice R, Rigano MM, et al.: **Antioxidants from Plants Protect against Skin Photoaging.** *Oxidative Med. Cell. Longev.* 2018; **2018**: 1454936.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 10. Zouboulis CC, Ganceviciene R, Liakou AI, et al.: **Aesthetic aspects of skin aging, prevention, and local treatment.** *Clin. Dermatol.* 2019; **37**(4): 365–372.
[PubMed Abstract](#) | [Publisher Full Text](#)
 11. Cömert ED, Mogol BA, Gökmen V: **Relationship between color and antioxidant capacity of fruits and vegetables.** *Curr. Res. Food Sci.* 2020 Jun 1; **2**: 1–10.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 12. Park S, Lim W, Bazer FW, et al.: **Quercetin inhibits proliferation of endometriosis regulating cyclin D1 and its target microRNAs in vitro and in vivo.** *J. Nutr. Biochem.* 2019 Jan; **63**: 87–100.
[PubMed Abstract](#) | [Publisher Full Text](#)
 13. Shafi W, Mansoor S, Jan S, et al.: **Variability in Catechin and Rutin Contents and Their Antioxidant Potential in Diverse Apple Genotypes.** *Mol. Basel Switz.* 2019 Mar 7; **24**(5): 943.
[Publisher Full Text](#)
 14. Rahmawati VA, Ramadan D, Anwar E, et al.: **Formulation and in vitro Penetration Test of Ethosomal Gel Extract of Malang Apple Peel (Malus sylvestris Mill) Dosage Form Contain Quercetin.** *Pullman Raja Orchid Hotel, Khon Kaen, Thailand.* 2016 [cited 2025 Jan 21].
[Reference Source](#)
 15. Das G, Paramithiotis S, Sundaram Sivamaruthi B, et al.: **Traditional fermented foods with anti-aging effect: A concentric review.** *Food Res. Int.* 2020 Aug 1; **134**: 109269.
[PubMed Abstract](#) | [Publisher Full Text](#)
 16. Tolosa L, Donato MT, Gómez-Lechón MJ: **General Cytotoxicity Assessment by Means of the MTT Assay.** *Methods Mol. Biol. Clifton NJ.* 2015; **1250**: 333–348.
[PubMed Abstract](#) | [Publisher Full Text](#)
 17. Arriola Benitez PC, Scian R, Comerici DJ, et al.: **Brucella abortus Induces Collagen Deposition and MMP-9 Down-Modulation in Hepatic Stellate Cells via TGF- β 1 Production.** *Am. J. Pathol.* 2013 Dec 1; **183**(6): 1918–1927.
[PubMed Abstract](#) | [Publisher Full Text](#)
 18. Tracy LE, Minasian RA, Caterson EJ: **Extracellular Matrix and Dermal Fibroblast Function in the Healing Wound.** *Adv. Wound Care.* 2016 Mar 1; **5**(3): 119–136.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 19. Barbieri JS, Wanat K, Seykora J: **Skin: Basic Structure and Function.** McManus LM, Mitchell RN, editors. *Pathobiology of Human Disease.* San Diego: Academic Press; 2014 [cited 2025 Jan 21]; pp. 1134–1144.
[Reference Source](#)
 20. Fernandes IR, Russo FB, Pignatari GC, et al.: **Fibroblast sources: Where can we get them?** *Cytotechnology.* 2016 Mar; **68**(2): 223–228.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 21. Addor FAS: **Antioxidants in dermatology.** *An. Bras. Dermatol.* 2017; **92**(3): 356–362.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 22. Curiel JA, Pinto D, Marzani B, et al.: **Lactic acid fermentation as a tool to enhance the antioxidant properties of Myrtus communis berries.** *Microb. Cell Factories.* 2015 May 7; **14**: 67.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 23. Li H, Huang J, Wang Y, et al.: **Study on the nutritional characteristics and antioxidant activity of dealcoholized sequentially fermented apple juice with Saccharomyces cerevisiae and Lactobacillus plantarum fermentation.** *Food Chem.* 2021 Nov 30; **363**: 130351.
[PubMed Abstract](#) | [Publisher Full Text](#)
 24. Zhang S, Hu C, Guo Y, et al.: **Polyphenols in fermented apple juice: Beneficial effects on human health.** *J. Funct. Foods.* 2021 Jan 1; **76**: 104294.
[Publisher Full Text](#)
 25. Chondrogianni N, Kapeta S, Chinou I, et al.: **Anti-ageing and rejuvenating effects of quercetin.** *Exp. Gerontol.* 2010 Oct; **45**(10): 763–771.
[PubMed Abstract](#) | [Publisher Full Text](#)
 26. Nurulita NA, Kusuma AM, Darsini D, et al.: **The Cytoprotective and Cell Recovery Properties of Apple Extracts on H2O2 induced-NIH3T3 Cells: An Anti Aging Candidate.** *Indones. J. Cancer Chemoprevention.* 2018 Jul 3; **9**(2): 78–85.
[Publisher Full Text](#)
 27. Shirzad M, Hamed J, Motevaseli E, et al.: **Anti-elastase and anti-collagenase potential of Lactobacilli exopolysaccharides on human fibroblast.** *Artif. Cells Nanomedicine Biotechnol.* 2018; **46**(sup1): 1051–1061.
[PubMed Abstract](#) | [Publisher Full Text](#)
 28. Chan LP, Tseng YP, Liu C, et al.: **Anti-oxidant and Anti-aging Activities of Fermented Vegetable-Fruit Drink.** *J. Food Nutr. Res.* 2021; **9**(5): 240–250.
[Publisher Full Text](#)
 29. Kang YM, Hong CH, Kang SH, et al.: **Anti-Photoaging Effect of Plant Extract Fermented with Lactobacillus buchneri on CCD-986sk Fibroblasts and HaCaT Keratinocytes.** *J. Funct. Biomater.* 2020 Jan 9; **11**(1): 3.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 30. Taofiq O, González-Paramás AM, Barreiro MF, et al.: **Hydroxycinnamic Acids and Their Derivatives: Cosmeceutical Significance, Challenges and Future Perspectives, a Review.** *Mol. J. Synth. Chem. Nat. Prod. Chem.* 2017 Feb 13; **22**(2): 281.
[Publisher Full Text](#)
 31. Ivarsson M, McWhirter A, Borg TK, et al.: **Type I collagen synthesis in cultured human fibroblasts: Regulation by cell spreading, platelet-derived growth factor and interactions with collagen fibers.** *Matrix Biol.* 1998 Feb 1; **16**(7): 409–425.
[PubMed Abstract](#) | [Publisher Full Text](#)
 32. Fligiel SEG, Varani J, Datta SC, et al.: **Collagen Degradation in Aged/ Photodamaged Skin In Vivo and After Exposure to Matrix Metalloproteinase-1 In Vitro.** *J. Invest. Dermatol.* 2003 May 1; **120**(5): 842–848.
[PubMed Abstract](#) | [Publisher Full Text](#)
 33. Varani J, Dame MK, Rittie L, et al.: **Decreased collagen production in chronologically aged skin: roles of age-dependent alteration in fibroblast function and defective mechanical stimulation.** *Am. J. Pathol.* 2006 Jun; **168**(6): 1861–1868.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 34. Rittié L, Fisher GJ: **Natural and sun-induced aging of human skin.** *Cold Spring Harb. Perspect. Med.* 2015 Jan 5; **5**(1): a015370.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 35. Pham QL, Jang HJ, Kim KB: **Anti-wrinkle effect of fermented black ginseng on human fibroblasts.** *Int. J. Mol. Med.* 2017 Mar; **39**(3): 681–686.
[PubMed Abstract](#) | [Publisher Full Text](#)
 36. Ro HS, Jang HJ, Kim GR, et al.: **Enhancement of the Anti-Skin Wrinkling Effects of Aloe arborescens Miller Extracts Associated with Lactic Acid Fermentation.** *Evid-Based Complement Altern. Med. ECAM.* 2020; **2020**: 2743594.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 37. Pokorný J: **Are natural antioxidants better – and safer – than synthetic antioxidants?** *Eur. J. Lipid Sci. Technol.* 2007; **109**(6): 629–642.
[Publisher Full Text](#)
 38. Lee PJ, Chen S: **Effect of adding ball-milled achenes to must on bioactive compounds and antioxidant activities in fruit wine.** *J. Food Sci. Technol.* 2016 Mar; **53**(3): 1551–1560.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 39. Hunaefi D, Akumo DN, Smetanska I: **Effect of Fermentation on Antioxidant Properties of Red Cabbages.** *Food Biotechnol.* 2013 Feb; **27**(1): 66–85.
[Publisher Full Text](#)
 40. Jin X, Chen W, Chen H, et al.: **Combination of Lactobacillus plantarum and Saccharomyces cerevisiae DV10 as Starter Culture to Produce Mango Slurry: Microbiological, Chemical Parameters and Antioxidant Activity.** *Mol. Basel Switz.* 2019 Nov 28; **24**(23): 4349.
[Publisher Full Text](#)
 41. Kruse CR, Singh M, Targosinski S, et al.: **The effect of pH on cell viability, cell migration, cell proliferation, wound closure, and wound reepithelialization: in vitro and in vivo study.**

Wound Repair Regen Off Publ Wound Heal Soc Eur Tissue Repair Soc. 2017 Apr; **25**(2): 260–269.

[Publisher Full Text](#)

42. Ricci A, Cirlini M, Calani L, *et al.*: **In vitro metabolism of elderberry juice polyphenols by lactic acid bacteria.** *Food Chem.* 2019 Mar 15; **276**: 692–699.

[PubMed Abstract](#) | [Publisher Full Text](#)

43. Shin D, Lee Y, Huang YH, *et al.*: **Probiotic fermentation augments the skin anti-photoaging properties of Agastache rugosa through up-regulating antioxidant components in UV-B-irradiated HaCaT keratinocytes.** *BMC Complement. Altern.*

Med. 2018 Jun 26; **18**(1): 196.

[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)

44. Wu C, Li T, Qi J, *et al.*: **Effects of lactic acid fermentation-based biotransformation on phenolic profiles, antioxidant capacity and flavor volatiles of apple juice.** *LWT.* 2020 Mar 1; **122**: 109064.

[Publisher Full Text](#)

45. Wardana M, Oktarina DAM, Pramana AAC: **Toxicology Study and MMP-1 and COL1A1 Expression of Malang Green Apple Juice Fermented by Lactobacillus plantarum DAD-13 and Saccharomyces cerevisiae Fermipan on Human Fibroblast: Potential Modality of Anti-aging.** *figshare.* 2025 [cited 2025 Jan 21].

[Publisher Full Text](#) | [Reference Source](#)

Open Peer Review

Current Peer Review Status: ? ?

Version 2

Reviewer Report 06 February 2026

<https://doi.org/10.5256/f1000research.191669.r452989>

© 2026 Suzauddula M. This is an open access peer review report distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.



Md Suzauddula Suzauddula

Kansas State University, Manhattan, KS, USA

Reviewer Comments

Title

1. The use of the term **"toxicology study"** is not fully justified by the experimental design. A toxicological evaluation generally requires parameters such as **IC₅₀ determination, defined safety thresholds, and/or mechanistic toxicity assessments**, which are not comprehensively addressed in the current manuscript.
2. The phrase **"Potential Modality of Anti-aging"** is too strong for the scope of this study. The work is **in vitro and exploratory**, and no direct measurements of antioxidant activity, functional skin aging models, or clinical relevance are included. Therefore, the title overstates the translational significance of the findings.

The authors are encouraged to revise the title accordingly. As a suggestion, the following revised title would be more accurate and appropriate:

"Effects of *Lactobacillus plantarum* DAD-13 and *Saccharomyces cerevisiae* Fermipan-fermented Malang green apple juice on MMP-1 and COL1A1 expression in human dermal fibroblasts: Implications for skin aging."

Introduction

1. While the authors discuss intrinsic and extrinsic aging, **genetic factors** are also a key contributor to intrinsic aging and should be explicitly mentioned. The introduction would benefit from inclusion of this aspect with **appropriate citation**.
2. Numerous **commercial chemical-based products** are currently used to improve skin tone and delay aging; however, many are associated with **side effects and long-term safety concerns**. Highlighting these limitations would help establish a clearer **research gap and rationale** for exploring natural and fermented products. The authors are encouraged to articulate this gap more clearly in the introduction.
3. The introduction would be strengthened by including **quantitative evidence** from previous studies demonstrating anti-aging effects of antioxidants derived from natural ingredients (e.g., percentage changes in collagen synthesis, reductions in MMP expression, or improvements in cell viability).

4. The manuscript states that fermentation can increase bioactive compounds, but this claim requires **supporting evidence**. The authors should cite **previous studies**, preferably involving apples or botanically similar fruits, that demonstrate fermentation-induced enhancement of antioxidant or bioactive content.

Methods

1. The authors should specify **the developmental stage of the green apples** used (e.g., ripe, mature) and describe the **selection criteria** (freshness, absence of physical damage or disease). The type of **food processor or blender** used should be mentioned. Additionally, the term *autoclave* is not appropriate without specifying **time, temperature, and pressure**, and may not be suitable for juice pasteurization. This should be clarified or revised.
2. It is assumed that the apple juice was **cooled after pasteurization** prior to microbial inoculation, as elevated temperatures would compromise microbial viability. This step should be explicitly described in the Methods section.
3. The source of the fibroblast cell line should be clearly stated, for example: "Human foreskin fibroblast cell lines were obtained from [source]."
4. In the description of cell culture conditions, the authors state that **10 mL FBS, 1 mL penicillin-streptomycin, and 1 mL amphotericin B** were added; however, the **total volume of DMEM** is not specified. The final medium composition should be clearly described.
5. In the MTT assay description, the sentence "*a stopper of 100 L SDS 10% in 0.1 N HCl*" is unclear. The unit should be corrected to **100 µL**, and the sentence revised for clarity.
6. The reported cell density of "**10,000 cells/mL**" appears to be incorrect. Please clarify whether this refers to **10,000 cells/mL or 10,000 cells per well**, and revise accordingly.
7. For **MMP-1 and COL1A1 quantification**, the authors should specify **the number of cells** used for RNA isolation and homogenization.
8. The **centrifugation temperature** used during RNA extraction (particularly during phase separation) should be stated to ensure reproducibility.

Discussion

1. The Discussion section currently repeats several results without sufficient interpretation. Instead, the authors should focus on **integrating their findings with previously published literature**. For example, studies showing that increased fibroblast viability and collagen production are indicative of anti-aging effects should be cited to support the study's conclusions.
 2. The statement that "*the decrease in MMP-1 levels at 125 µg/mL and the decrease in COL1A1 levels might indicate a decrease in living fibroblasts*" requires further explanation. The authors should clarify the biological significance of this observation and support it with **relevant literature** addressing dose-dependent cytotoxicity or stress responses in fibroblasts.
- Finally, although some references are included in the Discussion, they are often presented in **separate paragraphs rather than integrated into the interpretation of results**. The authors are encouraged to consistently support their arguments with appropriate citations throughout the Discussion, particularly in the first and second paragraphs.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

I cannot comment. A qualified statistician is required.

Are all the source data underlying the results available to ensure full reproducibility?

No source data required

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Bioactive compounds on aging and colon cancer.

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 29 January 2026

<https://doi.org/10.5256/f1000research.191669.r445126>

© 2026 Alugoju P. This is an open access peer review report distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.



Phaniendra Alugoju

Palacký University, Šlechtitelů, Olomouc, Czech Republic

The study entitled "Toxicology Study and MMP-1 and COL1A1 Expression of Malang Green Apple Juice Fermented by *Lactobacillus plantarum* DAD-13 and *Saccharomyces cerevisiae* Fermipan on Human Fibroblast: Potential Modality of Anti-aging" is interesting.

I recommend that the authors revise the title so that it more accurately reflects the main objective of the study.

Introduction

Please briefly summarize the current knowledge on the importance of fermented food products with anti-aging potency in the introduction.

Methods

Please use clear and more scientific subheadings (1 Green apple juice making and pasteurization, 2 Green apple juice fermentation, and 3 Green apple juice extraction) in the methods section.

Please use precise headings. Change "MMP-1 and COL1A1 quantification" to "Quantification of MMP-1

and COL1A1 mRNA by RT-qPCR” or “RT-qPCR Analysis of MMP-1 and COL1A1 Expression”
MTT assay: “...a stopper of 100 L SDS 10% in 0.1 N HCl” Correct 100 L to 100 µL SDS 10% in 0.1 N HCl”

Results

Please carefully write the results section. The authors showed the cell viability at different time points: 24, 48, and 72h. But in the methods section, it is mentioned that cell viability was checked only after 24 h. Please correct this.

What is the positive control used in this study?

Data analysis must be done carefully, and statistical differences should be reported accordingly. At 24 h, it seems that there is no significant difference between control and AJ/FAJ extracts. After 48 h, cell viability is increased beyond 100% in the negative control. Authors must normalize treatment groups' data to the negative control and then perform statistical analysis. Please specify how many fold differences were observed between treatment groups compared to the control. This applies to all figures and data. Analysis of the chemical composition of the extract is recommended.

Is the work clearly and accurately presented and does it cite the current literature?

Partly

Is the study design appropriate and is the work technically sound?

No

Are sufficient details of methods and analysis provided to allow replication by others?

Partly

If applicable, is the statistical analysis and its interpretation appropriate?

Partly

Are all the source data underlying the results available to ensure full reproducibility?

No source data required

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Cellular senescence and aging

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

The benefits of publishing with F1000Research:

- Your article is published within days, with no editorial bias
- You can publish traditional articles, null/negative results, case reports, data notes and more
- The peer review process is transparent and collaborative
- Your article is indexed in PubMed after passing peer review
- Dedicated customer support at every stage

For pre-submission enquiries, contact research@f1000.com

F1000Research