ÉLELMISZERVIZSGÁLATI KÖZLEMÉNYEK

JOURNAL OF FOOD INVESTIGATION

TUDOMÁNY - ÉLET - MINŐSÉG - BIZTONSÁG

LXX1. ÉVFOLYAM 2. SZÁM
VOL. 71, 2025 NO. 2

SCIENCE – LIFE – QUALITY – SAFETY

2025. JÚNIUS 30.
30 JUNE 2025

Examining Consumer Risk Perception in Genetically Modified Foods in Asia

A genetikailag módosított élelmiszerekkel kapcsolatos fogyasztói kockázat-értékelés vizsgálata Ázsiában

eviko.hu

TARTALOM – CONTENTS

ISSN 0422-9576

OPEN ACCESS

Itir ERKAN

DOI: https://doi.org./10.52091/EVIK-2025/2-1

Arrived: May 2025 – Accepted: June 2025

Examining Consumer Risk Perception In Genetically Modified Foods In Asia

Keywords: genetically modified food, risk perception, meta-analysis

1. Abstract

For many years, discussions on Genetically Modified (GM) Foods have left consumers in uncertainty. The aim of this study is to determine consumers' perception of the risks associated with genetically modified foods. A literature search was conducted in Web of Science, Pubmed, and Scopus between 2010 and 2024. Eight scientific studies in which consumers' risk perception was determined using this research were evaluated. The Jamovi 1.6.23 program was used for Meta-Analysis. In this context, correlation, heterogeneity, forest plot, funnel plot, and publication bias analyses were performed. In the evaluation, it was determined that the selected publications were suitable for meta-analysis. According to the studies that made up this meta-analysis, consumers have a negative and high overall risk perception of GMOs. The results might have been impacted by the small number of studies that were part of the meta-analysis and the participation of individuals from Asian nations in terms of cultural approach. However, the results indicate that in order to boost consumer trust, policymakers and decision-makers should create methods for transparent information. Additionally, a more thorough analysis of the personal and societal elements influencing risk perception is a crucial topic for further study. Consumer perceptions of GMO risk in the research analyzed for the meta-analysis are influenced by a number of factors, such as scientific ambiguity, environmental and health concerns, moral quandaries, and transparency challenges.

[1] Istanbul Yeni Yuzyil University, Faculty of Health Sciences, Department of Occupational Health and Safety

Itir ERKAN itir.erkan@yeniyuzyil.edu.tr http://orcid.org/ 0000-0002-5902-1936

Introduction

New genetic characteristics are given to a species by transferring genes from another living species and interfering with its existing genetic structure with gene technology. Scientists first discovered in 1946 that DNA can be transferred between organisms (Clive, 2011). Genetically modified organisms (GMOs) began to be discussed in the scientific community for the first time in the 1960s as a result of biotechnology advancements brought about by the discovery of recombinant deoxyribonucleic acid (rDNA) technology (Bitir et al., 2020). The first genetically modified (GM) plant was produced in 1983, using an antibiotic resistant tobacco plant (Magnuson et al., 1996).In particular, efforts are made to reduce agricultural costs, reduce chemical pesticides used to protect the crop, or increase the amount of product taken per unit area. By genetically modifying the seed, attempts are made to provide the seed with herbicide tolerance, insecticide, and virus resistance (Brookes, 2022). Common genetically modified crops are varieties of corn, soybeans, cotton, and canola.

GMO has been a current issue and a topic of discussion for many years. While some researchers emphasize its positive effects, others state that it has harmful effects (Erkan et al., 2014). This situation mostly affects consumers' perception of GMO risk through the media (Cui and Shoemaker, 2018). Values and personality traits have been demonstrated to influence a range of attitudes, such as those regarding immigration and political orientation, as was covered in the research review (Caprara et al., 2006; Vecchione et al., 2012). However, it has been discovered that one or the other is dominant, depending on the topic. The combined impact of these two variables on the perception of GM food risk has not yet been investigated. Studies show that personality factors may nearly entirely mitigate the impact of fundamental personal beliefs on risk perception when it comes to opinions on genetically modified foods.

The potential for cancer, teratogenesis, fatal consequences, and detrimental impacts on reproduction is the main concern raised by the contentious events and certain study findings on this subject. GM agriculture is now frequently examined in both positive and negative contexts and currently acts as a focal point of debate among the public and policymakers. Although there are several reports and evidence from human and animal studies on the potential health effects of GM food/feed, the evidence remains inconclusive, and public apprehensions have not been resolved (Domingo, 2016; Shen et al., 2022).

Consumer risk perception regarding genetically modified foods is important in terms of food safety, food regulations, and food risks. Therefore, research on consumer risk perception is needed. Asian countries, besides encompassing a large portion of the world's population, are a region where biotechnology-based agricultural practices are rapidly spreading and where public debate is intense. Approaches to the production, import, labeling, and consumption of GMO products vary (Amirhusin, 2023). The objective of this study is to determine Asian consumers' risk perception towards GMO products using meta-analysis method.

Materıal And Methods

In this study, articles examining consumers' risk perception about genetically modified foods were examined using the meta analysis method. Figure 1 presents the flow diagram of the applied search and inclusion strategy.

Characteristics of the Sample

A literature search was conducted in Web of Science, Pubmed, and Scopus between 2010 and 2024. The search was restricted to English and full-text research articles. The search was performed for combinations of keywords related to GM technology and biological risk perception. The search keywords included the following 'genetically modified', 'GMO', 'GM', 'food' 'risk perception', 'biological risk', 'survey', 'consumer'. This study is a quantitative study. In the analysis of these articles, the following inclusion criteria were applied: (1) the subject and the articles of the study explicitly focused on GMO risk perception, (2) the article presents previously unpublished quantitative data (exclusions: review articles and data from conference presentations), (3) the sample consisted of consumers. It was determined that all articles were from consumers in Asian countries. Therefore, there was no need to impose regional restrictions.

Analysis via the Jamovi program

The Jamovi 1.6.23 program was used for meta-analysis. The study data was first transferred to a file created in the Excel program, tables were created in this file and then analyzed in the Jamovi program. Compilations of research conducted using keywords determined in Web of Science, Pubmed and Scopus, research published in languages other than English, and research with no specified year range or keywords were excluded. The analysis was carried out using the Fisher r-to-z transformed correlation coefficient as the outcome measure. A random-effects model was fitted to the data.

Heterogeneity

The amount of heterogeneity (i.e. tau²), was estimated using the restricted maximum-likelihood estimator (Viechtbauer, 2005). In addition to the estimate of tau², the Q-test for heterogeneity (Cochran, 1954) and the I^2 statistic are reported. In case any amount of heterogeneity is detected (i.e. $tau^2 > 0$, regardless of the results of the Q-test), a prediction interval for the true outcomes is also provided. Studentized residuals and Cook's distances are used to examine whether studies may be outliers and/or influential in the context of the model. Studies with a studentized residual larger than the 100 x (1 - 0.05/(2 x n)) percentile of a standard normal distribution are considered potential outliers (i.e. using a Bonferroni correction with two-sided alpha = 0.05 for n studies included in the meta-analysis). Studies with a Cook's distance larger than the median plus six times the interquartile range of the Cook's distances are considered to be influential.

Funnel plot, publication bias, forest plot test

The rank correlation test and the regression test, using the standard error of the observed outcomes as predictor, are used to check for funnel plot asymmetry. Forest plot, publication bias assessment, and funnel plot analysis were performed in the Jamovi program.

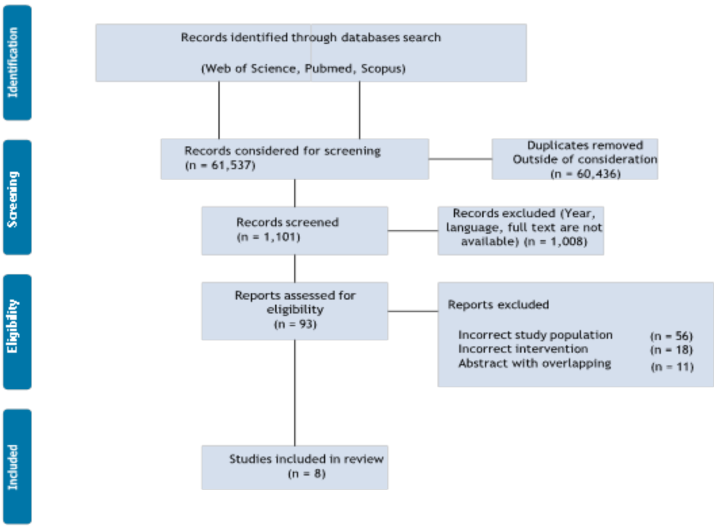


Figure 1 Meta-analysis flow diagram

Results And Discussion

It was determined that the risk perceptions of the participants in the studies evaluated in the study were evaluated through surveys. Table 1 shows information about the articles evaluated in the meta-analysis study. The total number of participants was determined as 6,057.

Table 1 Information on the articles evaluated in the meta-analysis study

Article	Authors	Publication Year	Participants' countries	N	Polit&Bck point
Food-related hazards in China: consumers' perceptions of risk and trust in information sources	Liu R., Pieniak Z. & Verbeke W	2014	China	971	11/12
The effects of epistemic trust and social trust on public acceptance of genetically modified food: An empirical study from China	Hu L, Liu R, Zhang W & Zhang T	2020	China	1,091	11/12
Consumer-perceived risks of genetically modified food in China	Xu, R., Wu, Y., & Luan, J.	2020	China	504	10/12
Exploring factors affecting the acceptance of genetically edited food among youth in Japan	Farid, M., Cao, J., Lim, Y., Arato, T., &Kodama, K.	2020	Japan, China, Korea	180	9/12
The influence of consumers' knowledge on their responses to genetically modified foods	Hwang, H., & Nam, S. J.	2021	Korea	1000	11/12

Article	Authors	Publication Year	Participants' countries	N	Polit&Bck point
How does information exposure affect public attitudes toward GMO in China? The mediating and moderating roles of conspiracy belief and knowledge	Du Z, Yuqi Xiao Y &Xu J	2022	China	518	10/12
Consumer responses to genetically modified food in China: The influence of existing general attitudes, affect and perceptions of risks and benefits	Jin S, Li W, Dawson I.G, Clark, B., Chen, S., & Frewer, L. J.	2022	China	1,411	11/12
Understanding Thai consumers' ıntentions to purchase genetically modified foods	Zhu, B., Phunthasaen, A., Rungruengarporn, C., & Pinpak, S.	2024	Thailand	382	10/12

For the remaining eight publications at the end of the study, 12 criteria, which were proposed by Polit and Beck, among the criteria for evaluating research quality were used. These criteria allow for an overall assessment of the objectives, sample characteristics, findings, and results of the studies. Each study was evaluated on all criteria and separately by the researchers, and if it meets each item, it is given a '0 point' value if it does not meet '1 point'. The scores that the study can get according to the criteria range from 0 to 12 (Polit and Beck, 2009).

Table 2 Correlation coefficients

Random-Effects Model (n = 8)						
	Estimate	se	Z	p	CI Lower Bound	CI Upper Bound
Intercept	-0.773	0.166	-4.67	<.001	-1.098	-0.449
	.	.	.	.	.	.
Not. Tau² Estimator: Restricted Maximum-Likelihood						

Table 3 Heterogeneity statistics

Tau	Tau²	I²	H²	R²	df	Q	p
0.422	0.1783 (SE= 0.1171)	82.29%	5.648	.	7.000	39.309	<.001

Correlation analysis

A total of n=8 studies were included in the analysis. The observed Fisher r-to-z transformed correlation coefficients ranged from -0.029 to -1.472, with the majority of estimates being negative (100%). This study, based on a random effects meta-analysis of eight studies, showed that the overall effect of the examined variable was statistically significant (b= -0.77; 95% CI [-1.10, -0.45]; p < 0.001). However, considering the high heterogeneity (I^2= 82.29%) and indicators of publication bias (Egger p = 0.004; Kendall tau p = 0.006), this effect size can vary significantly from study to study, potentially limiting generalizability.

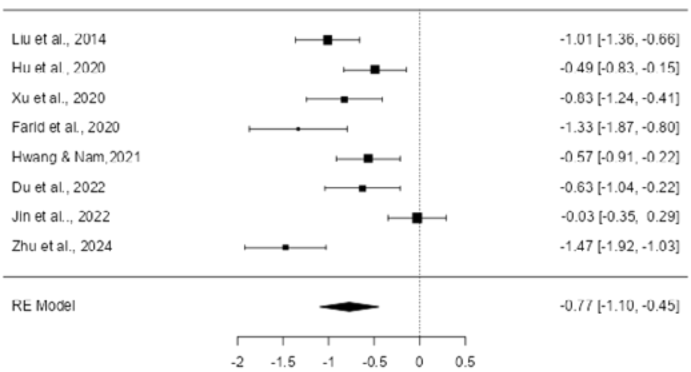


Figure 2 Forest plot

Forest plot analysis

The findings of eight studies examining consumer risk perception regarding GMOs. According to the results obtained from the random effects model, the overall effect size was -0.77 (95% CI: -1.10, -0.45), indicating that consumers have a significantly negative and high risk perception toward GMOs. The fact that the confidence interval does not cross zero confirms that the result is statistically significant. While most studies produced negative and significant results (e.g., Liu et al., 2014; Xu et al., 2020; Farid et al., 2020; Zhu et al., 2024), only the finding reported by Jin et al. (2022) was found to be non-significant (Fig. 2). This may be explained by cultural contexts in different countries, consumers' knowledge and awareness levels, and the diversity of information received from the media and public authorities. Indeed, consumers' lack of knowledge and conspiracy beliefs about GMOs have been reported as factors that increase risk perception in the previous literature.

Table 4 Publication Bias Assessment

Test Name	value	p
Fail-Safe N	327.000	<0.001
Kendalls Tau	-0.786	0.006
Egger's Regression	-2.910	0.004

Note. Fail-safe N Calculation Using the Rosenthal Approach

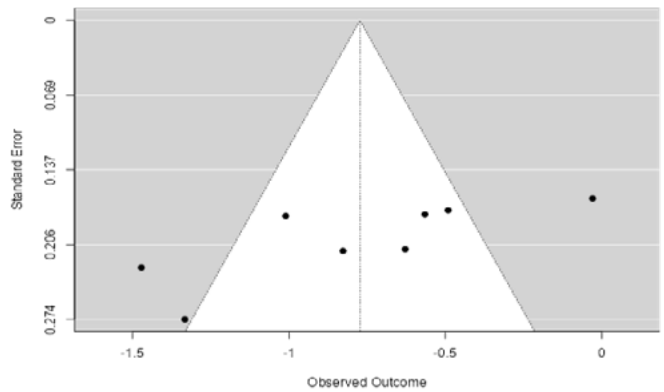


Figure 3 Funnel plot

In order to determine the publication bias, Table 4 is examined, and the results of the publication bias test applied to the study data are given. Another method used for publication bias is the funnel plot; the data should be spread equally in both parts of the graph (Bakioglu and Ozcan, 2016). Egger's regression test was partially statistically significant ($t = -2.910$, $p = 0.004$). This finding suggests a high probability of publication bias among the studies included in the meta-analysis. In other words, studies reporting neutral or low consumer risk perception regarding GMOs were not sufficiently reported in the literature, while studies emphasizing negative risk perceptions were more frequently published. However, the number of studies (less than 10) limits the possibility of a definitive the Egger regression assessment. When the funnel plot is examined, it is seen that the distribution of studies is not completely symmetrical, and that there are more studies reporting negative results (Figure 3). This indicates publication bias.

This study found that the majority of publications examining consumer risk perception focused on Chinese consumers, while other publications focused on Japanese, Korean, and Thai consumers. Because all of the studies were conducted with consumers in Asia, the results are believed to be inconsistent with the overall picture. It is anticipated that research conducted in different geographical settings could yield different results. However, the meta-analysis's search criteria eliminated other publications, yielding only eight articles for review. The findings of this study are consistent with previous studies demonstrating that consumers in Asian countries have a high-risk perception and negative attitudes toward GMO foods. Considering the cultural roots of this situation, it can be argued that the collectivist nature of Asian societies leads individuals to make decisions not only considering their own health but also that of society as a whole. Furthermore, past food safety incidents have weakened consumer confidence in food technologies, leading to a more cautious and cautious approach toward GMOs. The strong traditional culinary culture and understanding of naturalness in countries such as Japan, Korea, China, and India also contribute to the negative perception of GMOs. Furthermore, religious and ethical sensitivities, as well as the stringency of regulatory policies, reinforce this perception of risk. Therefore, the high risk perception toward GMOs in Asian countries stems not only from a lack of scientific knowledge but also from cultural values, social memory, and trust. (Chowdhury et al., 2024; Campos et al., 2024). Therefore, it is believed that conducting regional research rather than global research will enable policymakers to develop region-specific strategies and regulate GMO labeling and public health policies accordingly.

In a study conducted by Liu et al. (2014), the findings are as follows: Three categories were used to group the participants: 18.2% were moderately concerned, 21.7% were anxious and uninformed, and 60.1% were anxious and informed. The first two groups had a high degree of personal risk perception and anxiety about foodborne hazards in China. Poor-quality food and counterfeit food were the two risks they were most worried about. The three information sources that people utilized most often to learn about food safety were personal communication, the Internet, and television. Magazines, books, and brochures were used less frequently by worried and ignorant customers than by other customers. According to the first two consumer groups, research institutes, personal experience, and medical professionals were the three most reliable sources of information. Only themselves and medical professionals were highly trusted by consumers who were moderately concerned. Consumer perceptions of the levels of the knowledge, honesty, and relevance of different information sources significantly and positively influenced their trust in these sources.

In a study conducted in 2020, three major findings were obtained: First, the acceptance of GM food is significantly influenced indirectly by epistemic trust, which is a key antecedent of perceived risks and benefits. Second, perceived benefits are positively impacted by trust in public organizations, but perceived hazards are negatively impacted by trust in industrial groups. Thirdly, confidence in public organizations did not have a substantial direct effect on perceived dangers, while trust in industrial organizations did not exert a large direct effect on perceived advantages, despite popular belief. As a result, the adoption of GM food is influenced differently by trust in public and industrial entities (Hu et al., 2020).

Consumers' objective knowledge level determines which groups in the sample are familiar with and which are unfamiliar with them. The findings indicate that consumers' perceived risks of GMO foods are significantly reduced when additional information is provided, and that positive consumer attitudes toward GMO technology have a significant negative impact on these risks only for those unfamiliar with the technology. The perceived dangers of GMO foods are largely unaffected by consumers' faith in the government, biotechnology researchers, the media, or food companies. Consumers' lack of trust in GMO food stakeholders is likely the root of this. Consumers believe there is too little information available to them explaining the benefits and drawbacks of GMO foods. Furthermore, consumers have little objective understanding of GMO foods (Xu et al., 2020).

To understand the factors that may influence the adoption of genetically modified foods among young people in Japan, a survey was conducted among 180 Japanese university students by Farid et al. Approximately 24% of participants stated that they were not concerned about purchasing GMO foods, but this figure increased to 41% after being informed. This result indicated that risk perception decreased after being informed (Farid et al., 2020).

A study by Hwang & Nam found that consumers' perceived risk levels varied depending on the type of information they received. Participants in the study were divided into three groups: those who perceived their knowledge level to be high, those who assessed their knowledge level realistically, and those who assessed their knowledge level inadequately. Participants who perceived their level of knowledge to be high were found to have the highest risk perception. Those with higher education and income levels were found to have higher risk perception (Hwang & Nam, 2021).

A study by Du and colleagues found that consumers' perception of risk regarding GMOs increased with information obtained from unofficial sources. This negative news led to an increase in consumers' perception of risk toward GMO products. Therefore, the article discusses the impact of information obtained from official media outlets and government-supported publications on changing the risk perception towards GMOs into a positive attitude (Du et al., 2022).

When participants associated GMO practices with tampering with nature, perceived unnaturalness and risk perceptions increased. Collectively, the results suggest that communication design regarding GMO food practices should incorporate the impact evoked by the practices' reported benefits and risks, and also consider the potential influence of recipients' existing general attitudes (Jin et al., 2022).

A study by Zhu and colleagues found that consumers with higher knowledge and awareness about GMOs had lower risk perception. Highly educated individuals had a lower risk perception than those with lower education (Zhu et al., 2024).

Previous studies show that consumers have more positive attitudes towards GMOs in specific areas, such as their medical applications (e.g., insulin production) and environmental benefits (e.g., reducing pesticide use), and the perception that they can be a solution to the hunger problem (Frewer et al., 1997; Costa-Font et al., 2008; Erkan and Dastan, 2017).

In a consumer survey conducted in China, 46.7% of the respondents had a negative view of GMO foods, while only 11.7% said they understood the basic principles of GMO technology. Most consumers (69.3%) obtain their information from the Internet and think that the media mostly reports negative news about GMOs (64.3%). This shows that consumers are skeptical about GMOs due to the lack of information and media influence (Cui and Shoemaker, 2018).

In the research conducted in 2020, which examined 522 Twitter accounts, it was determined that personality traits would strongly moderate the effect of individual values on risk perception (Whittingham et al.,2020).

In a Pew Research Center survey conducted in 20 countries between 2019 and 2020, 48% of respondents (median of 20 countries) believe that consuming GMO foods is unsafe. Consumers view GMOs as an 'unnatural' intervention, and this perception is particularly pronounced in countries such as Russia (70%) and Italy (62%). This reflects psychological concerns that GMOs interfere with biological processes. It has also been found that individuals with higher levels of education (especially those who have taken science courses) tend to find GMOs safer. For example, in the Netherlands, 50% of individuals who have taken at least three science courses find GMOs safe, while this rate drops to 27% among those with less science education. This suggests that higher levels of education reduce the perception of risk (Pew Research Center, 2020).

According to a study conducted in Türkiye, some participants pointed out the positive aspects of GMOs such as vaccines, drug production, and resistance of plants to pests. Some health workers have a positive approach to the medical and agricultural benefits of GMOs (Soyler et al., 2021).

A study conducted in 2022 suggested that consuming GMOs might be linked to significant negative health outcomes, including heightened mortality rates, tumors or cancers, and reproductive issues. Given these scientific revelations, consumers are worried about the potential long-term health impacts of GMO products, especially regarding organ toxicity (specifically to the liver and kidneys) and the potential for allergic reactions. Additionally, ecological risks such as gene escape and a decrease in biodiversity are raising alarms among consumers (Shen et al.,2022).

A study published in Frontiers in 2024 states that negative information about GMOs spread through social media and the media increases consumers' perception of risk. A survey conducted in the Czech Republic found that lack of information about GMOs and discussions on social media lead consumers to see GMOs as morally problematic. However, it is stated that information campaigns can change this perception positively (Cabelkova, 2024).

The studies included in this meta-analysis indicate that consumers' overall risk perception of GMOs is high and negative. The limited number of studies included in the meta-analysis ((language, database, period, etc. reasons)and the presence of participants from Asian countries in terms of cultural approach may have affected the findings. Nevertheless, the findings suggest that policymakers and decision-makers should develop transparent information strategies to increase consumer confidence. Since consumers from Asian countries participated in all of these studies, the results cannot be generalized. Furthermore, a more in-depth examination of the cultural and individual factors shaping risk perception is an important area for future research.

Conclusion

In the studies reviewed for the meta-analysis, consumer perceptions of GMO risk stem from a variety of factors, including scientific uncertainty, health and environmental concerns, ethical dilemmas, and transparency issues. Although many advocate for clearer labeling to inform consumers, conflicting studies and unknown long-term effects contribute to public concern. These uncertainties negatively impact consumer risk perception, highlighting the need for comprehensive research and clear communication to allay fears about genetically modified organisms. Additionally, it was concluded that the perception that accurate information is provided to consumers by government officials and media organizations will affect the risk perception towards GMO products.

References

Amirhusin, B. (2023). *Genetically modified organism status, regulation, approval, labeling, and consumer perception in ASEAN.* M. A. Nawaz, G. Chung, K. S. Golokhvast & A. M. Tsatsakis (Eds): In GMOs and political stance (pp. 129-150). Academic Press.

Bakioglu, A. & Ozcan, S. (2016). *Meta Analysis.* Nobel Academic Publishing, 1st ed., Istanbul, Turkiye.

Bitir B, Erkan I & Yukseloğlu E H, Searching of the genetically modified organisms and their products' status and evaluation of food safety and regulations in Turkey in terms of the Forensic Sciences. *Commagene Journal of Biology,* 4 (2) (2020) 104-109.

Brookes, G. (2022). Genetically modified (GM) crop use 1996–2020: Environmental impacts associated with pesticide use change. *GM Crops & Food,* 13(1), 262-289.

Cabelkova, I., Sanova, P., Hlavacek, M., Broz, D., Smutka, L., & Prochazka, P. (2024). The moderating role of perceived health risks on the acceptance of genetically modified food. *Frontiers in Public Health,* 11, 1275287.

Campos, R. D. C. L., Boas, L. H. D. B. V., & Brito, M. J. D. (2024). Food safety, consumption behavior, and personal values: an integrative review. Revista de Administração da UFSM, 17(3), e4.

Caprara, G., S. H. Schwartz, C. Capanna, M. Vecchione, and C. Barbaranelli. 2006. "Personality and Politics: Values,Traits, and Political Choice." Political Psychology 27 (1): 1–28. doi:10.1111/j.1467-9221.2006.00447.x.

Cochran, W. G. (1954). The combination of estimates from different experiments. Biometrics, 10,101–129.

Chowdhury, S., Ahmed, M. T., Oni, F. A., & Mamun, T. M. (2024). Individualistic or collectivistic: which consideration motivates purchasing intention of organic foods? A developing country perspective. *Journal of Agribusiness in Developing and Emerging Economies,* 14(5), 1089-1108.

Clive J (2011). *Global status of commercialized Biotech/GM crops.* ISAAA Briefs 43. International Service for the Acquisition of Agri-biotech Applications, Ithaca

Cui, K.,& Shoemaker, S. P. (2018). Public perception of genetically-modified (GM) food: a nationwide Chinese consumer study. *npj Science of Food,* 2(1), 10.

Domingo, JL (2016) Safety assessment of GM plants: an updated review of the scientifc literature. *Food Chemistry Toxicology*,95:12–18.

Du, Z., Xiao, Y., & Xu, J. (2022). How does information exposure affect public attitudes toward GMO in China? The mediating and moderating roles of conspiracy belief and knowledge. *Frontiers in Psychology,* 13, 955541.

Erkan, I., Kolusayin, MO., & Yukseloglu, EH. (2014). Forensic genetic examinations of genetically modified organisms, *Turkish Journal of Forensic Science,* 13(3): 47-50.

Erkan, I. & Dastan K. (2017). Real-time PCR detection of genetically modified organisms in several food products and their environmental effects in Turkey, *Fresenius Environmental Bulletin,* 26(4): 2589-2595.

Farid, M., Cao, J., Lim, Y., Arato, T., & Kodama, K. (2020). Exploring factors affecting the acceptance of genetically edited food among youth in Japan. *International Journal of Environmental Research and Public Health*, 17(8), 2935.

Hu, L., Liu, R., Zhang, W., & Zhang, T. (2020). The effects of epistemic trust and social trust on public acceptance of genetically modified food: An empirical study from China. *International Journal of Environmental Research and Public Health*, 17(20), 7700.

Hwang, H.,& Nam, S. J. (2021). The influence of consumers' knowledge on their responses to genetically modified foods. GM Crops & Food, 12(1), 146-157.

Jin, S., Li, W., Dawson, I. G., Clark, B., Chen, S., & Frewer, L. J. (2022). Consumer responses to genetically modified food in China: The influence of existing general attitudes, affect and perceptions of risks and benefits. *Food Quality and Preference,* 99, 104543.

Liu, R., Pieniak, Z., & Verbeke, W. (2014). Food-related hazards in China: Consumers' perceptions of risk and trust in information sources. *Food Control,* 46, 291-298.

Magnuson, N. S., Linzmaier, P. M., Gao, J. W., Reeves, R., An, G., & Lee, J. M. (1996). Enhanced recovery of a secreted mammalian protein from suspension culture of genetically modified tobacco cells. *Protein Expression and Purification,* 7(2), 220-228.

Pew Research Center (2020). https://www.pewresearch.org/short-reads/2020/11/11/many-publics-around-world-doubt-safety-of-genetically-modified-foods/

Polit, D. F.,& Beck, C. T. (2009). *Literature reviews: Finding and reviewing research evidence.* In: D. F. Polit, & B. C. Tatano (Eds.), Essentials of Nursing Research: Appraising Evidence for Nursing Practice.7th ed. (pp.169-193). Philadelphia: Lippincott Williams & Wilkins.

Shen, C., Yin, X. C., Jiao, B. Y., Li, J., Jia, P., Zhang, X. W., Cheng, X. H., Ren, J. X., Lan, H. D., Hou, W. B., Fang, M., Li, X., Fei, Y. T., Robinson, N., & Liu, J. P. (2022). Evaluation of adverse effects/events of genetically modified food consumption: a systematic review of animal and human studies. *Environmental Sciences Europe,* 34, 8.

Soyler, N., Ipar, M.S., Kocatepe, D. (2021). Determination of the genetically modified organism (GMO) awareness levels of hospital workers: example of Sinop. Food and Health, 7(1), 1-14.

Whittingham, N., Boecker, A., & Grygorczyk, A. (2020). Personality traits, basic individual values and GMO risk perception of twitter users. Journal of Risk Research, 23(4), 522-540. https://doi.org/10.1080/13669877.2019.1591491

Xu, R., Wu, Y., & Luan, J. (2020). Consumer-perceived risks of genetically modified food in China. *Appetite*, 147, 104520.

Vecchione, M., G. Caprara, H. Schoen, J. L. Castro, and S. H. Schwartz (2012). The Role of Personal Values and BasicTraits in Perceptions of the Consequences of Immigration: A Three-Nation Study. *British Journal of Psychology* 103 (3): 359–377. doi:10.1111/j.2044-8295.2011.02079.x.

Viechtbauer, W. (2005). Bias and efficiency of meta-analytic varianceestimators in the random-effects model. *Journal of Educational and Behavioral Statistics,* 30(3), 261–293.

Yigit, A. (2020). A Research on the Determination of Hospital Efficiency by Meta Analysis Method, *SDU Journal of Health Sciences*, 11(1): 24-32.

Zhu, B., Phunthasaen, A., Rungruengarporn, C., & Pinpak, S. (2024). Understanding Thai Consumers' Intentions to Purchase Genetically Modified Foods. Research on World Agricultural Economy, 5(4).

Itir ERKAN

DOI: https://doi.org./10.52091/EVIK-2025/2-1

Érkezett: 2025 május – Elfogadva: 2025 június

A genetikailag módosított élelmiszerekkel kapcsolatos fogyasztói kockázatértékelés vizsgálata Ázsiában

Kulcsszavak: génmódosított élelmiszerek, kockázatérzékelés, metaanalízis

1. Absztrakt

A génmódosított élelmiszerekkel kapcsolatos viták évek óta bizonytalanságban tartják a fogyasztókat. E tanulmány célja annak meghatározása, hogy az ázsiai fogyasztók hogyan érzékelik a génmódosított élelmiszerekkel kapcsolatos kockázatokat. A Web of Science, a Pubmed és a Scopus adatbázisok alapján 2010 és 2024 között végeztünk irodalomelemzést. A kutatás során nyolc olyan tudományos tanulmányt értékeltünk, amelyekben a fogyasztók kockázati percepcióját határozták meg. A metaanalízishez a Jamovi 1.6.23 programot használtuk. Ennek során korrelációs, heterogenitási, erdődiagram-, tölcsérdiagram- és publikációs torzítás-vizsgálatokat végeztünk. Az értékelés során megállapítottuk, hogy a kiválasztott publikációk alkalmasak meta-elemzésre. A meta-elemzést alkotó tanulmányok szerint a fogyasztók negatív és magas általános kockázati percepcióval rendelkeznek a GMO-kkal kapcsolatban. Az eredményeket befolyásolhatta a meta-elemzésben szereplő tanulmányok kis száma, valamint az ázsiai országokból származó személyek kulturális megközelítése. Az eredmények azt is mutatják, hogy a fogyasztói bizalom növelése érdekében a politikai és más döntéshozóknak átlátható információszolgáltatási módszereket kell kidolgozniuk. Ezenkívül a kockázatérzékelést befolyásoló személyes és társadalmi tényezők alaposabb elemzése is fontos téma a további kutatások számára. A metaanalízis során vizsgált kutatási eredményekben a fogyasztók GMO-kkal kapcsolatos kockázatérzékelését számos tényező határozza meg, például a tudományos bizonytalanság, a környezeti és egészségügyi aggályok, az erkölcsi dilemmák és az átláthatósági kihívások.

[1] Isztambuli Yeni Yuzyil Egyetem, Egészségtudományi Kar, Munkaegészségügyi és Biztonsági Tanszék

Itir ERKAN itir.erkan@yeniyuzyil.edu.tr http://orcid.org/ 0000-0002-5902-1936

Kalaiselvan DHANUJA[1] – Vellingiri VADIVEL[1] ✉

DOI: https://doi.org./10.52091/EVIK-2025/2-2

Arrived: May 2025 – Accepted: June 2025

Effect of Phytochemicals on Food-Borne Pathogenic Bacteria, Oxidative Spoilage and Shelf Life of Pasteurized Cow Milk

Keywords: Milk spoilage; Phytochemicals; Antimicrobial; Antioxidant; Foodborne pathogens

1. Abstract

Milk is highly vulnerable to spoilage caused by microbial contamination and oxidation. These spoilage processes highlight the need for effective preservation methods to extend milk's shelf life while maintaining its nutritional integrity. In this context, phytochemicals with antibacterial and antioxidant properties could serve as useful agents to prevent microbial and oxidative spoilage in milk. The objective of this project was to screen phytochemicals such as catechin, quercetin, gallic acid, sinapic acid, betanin, and glycyrrhetinic acid for their antibacterial activity against *E. coli* and *L. monocytogenes*. Additionally, we aimed to assess the effectiveness of the selected compounds in extending the shelf life of pasteurized milk. The results indicated that gallic acid demonstrated significant antibacterial activity, with a zone of inhibition (ZOI) ranging from 18.66 to 13.00 mm and a minimum inhibitory concentration (MIC) between 18.37 and 36.74 mM. Consequently, gallic acid was chosen for further investigation of its antibacterial mechanisms through scanning electron microscopy (SEM) analysis. The antioxidant activity of gallic acid was assessed using the DPPH assay, and its ability to inhibit the oxidation of lipids and proteins in milk was studied. Additionally, the sensory profile, total bacterial count, and levels of malondialdehyde (MDA) and protein carbonyls were evaluated in pasteurized milk treated with gallic acid stored for 4 days at 4 °C. It was observed that gallic acid damages the membrane structure of food pathogens such as *E. coli* and *L. monocytogenes*, as visualized through scanning electron microscopy (SEM). The DPPH radical scavenging activity was found to have an IC-50 value of 43 µg/mL, with gallic acid demonstrating a 48.21% inhibition of induced lipid peroxidation and a 6.78% inhibition of protein oxidation. On the fourth day of treatment, gallic acid significantly reduced the colony-forming units (CFU) in milk to 5.71 $\log_{10}$ CFU/mL, compared to 6.17 $\log_{10}$ CFU/mL in the potassium dichromate group. Furthermore, the inhibition levels of protein carbonyls (1.33-fold) and MDA (2.06-fold) were significantly higher in gallic acid treated milk than in - samples treated with potassium dichromate. Considering these factors, gallic acid could be an effective, safe, and environmentally friendly preservative for the dairy industry.

[1] Chemical Biology Lab (ASK-II-409), School of Chemical and Biotechnology, SASTRA Deemed University, Thanjavur, Tamilnadu, India
✉ Corresponding author

Kalaiselvan DHANUJA		https://orcid.org/0009-0009-3850-104X
Vellingiri VADIVEL	vadivel@carism.sastra.edu	https://orcid.org/0000-0002-2709-433X

2. Introduction

Milk, often called nature's elixir, is a nutrient-rich liquid obtained from animals such as cows, buffalo, and goats for human consumption. Its nutritional profile makes milk an essential part of a well-balanced diet, providing a variety of vital nutrients that contribute to overall health and well-being (Smith et al., 2022). It is particularly known for its high content of protein (3.2 g per 100 g), fat (3.6 g per 100 g), lactose (4.7 g per 100 g), calcium (122 mg per 100 g) and phosphorus (119 mg per 100 g). Additionally, milk contains essential vitamins, including vitamin A (126 IU), vitamin D (2 IU) and riboflavin (0.17 mg per 100 g), which play a significant role in various metabolic processes as physiologically active coenzymes (Pereira, 2014).

Milk production is a thriving industry worldwide, with various countries contributing to the global supply. India ranks first among the top milk producing nations, accounting for approximately 221 million tonnes of the total world supply of 930 million tonnes (FAO, 2022). According to the Basic Animal Husbandry Statistics (BAHS, 2022), Tamil Nadu produced 10.10 million tonnes of milk in the 2021–2022 period, representing 4.57% of the total milk production of the nation. The dairy sector also provides employment opportunities and contributes to rural development (Hanrahan et al., 2018). However, it is estimated that 55 million tonnes of milk were disposed of worldwide in 2018 due to spoilage during production and distribution (Gross, 2018). In India, a significant volume of milk — 1,500 litres — processed in the Aavin Government dairy unit in Tamil Nadu was spoilt (The New Indian Express, 2024).

Milk is a perishable product that is susceptible to microbial contamination and chemical oxidation, leading to spoilage (Saha et al., 2024). Microorganisms, such as bacteria, can contaminate milk, leading to spoilage and posing potential health risks to consumers. The presence of food pathogens such as Staphylococcus aureus, *Salmonella enterica* serovar *Typhi*, *Listeria monocytogenes*, *Escherichia coli*, *Clostridium botulinum* and *Bacillus cereus*, which all not only affect the taste and odor of milk, but also pose health risks (Kabiraz et al., 2023). One significant cause of milk spoilage is the oxidation of unsaturated fatty acids, which is triggered by reactive oxygen species (ROS) (Soares et al., 2009). This oxidation leads to the formation of lipid hydroperoxides, propanal, hexanal, 4-hydroxynonenal (4-HNE), and malondialdehyde (MDA) (Kapusta et al., 2018). Spoilage microorganisms not only contribute to the direct deterioration of milk but also create favorable conditions for the growth and spread of pathogenic species. This occurs through various mechanisms, including alterations in pH, nutrient release, and a reduction in natural microbial competition, all of which enhance the survival and reproduction of pathogens (Mafe et al., 2024; Martín-Miguélez et al., 2025). Given these factors, it is essential to evaluate the antimicrobial effects of selected compounds against pathogenic bacteria to ensure effective spoilage control and overall food safety.

Although physical methods such as refrigeration, pasteurization, and ultraviolet irradiation are effective at inhibiting in microbial growth and delaying oxidation in milk on an industrial scale (Machado et al., 2017; Zheng et al., 2024), they have several drawbacks. These methods often consume a lot of energy, provide only limited shelf life extension, and can negatively impact the sensory qualities and nutritional profile of milk. For example, high-temperature pasteurization can lead to loss of nutrients, alter the taste of milk, and may not always eliminate all pathogens (Coutinho et al., 2018). Furthermore, chemical treatments, such as preservatives like potassium dichromate, sodium bicarbonate, and hydrogen peroxide, are also used to prevent spoilage in milk. However, these chemicals can have negative health effects and may change the taste and nutritional value of the milk (Upadhyay et al., 2014). Potassium dichromate is being used in milk preservation at 0.06% in industries (Zajac et al., 2016), but it is accused of containing hexavalent chromium, an irritant that known to cause nosebleeds, ulcers, rhinitis, allergic dermatitis, and is carcinogen (Kroger, 1985; Schecter et al., 2009). In this study, potassium dichromate was included solely for a comparative evaluation of its preservative efficacy and is not recommended for food use. Its application in food is not allowed under current regulatory guidelines.

Due to the limitations and drawbacks of existing physical and chemical preservation methods, there is a growing interest in exploring alternative natural preservatives for milk. Natural extracts, such as those from fig and olive leaves, have been reported to effectively preserve milk (Abdel-Aziz et al., 2020). However, plant extracts often suffer from variability in the concentration of active compounds (Beya et al., 2021), which can lead to inconsistencies in industrial applications. Natural compounds such as vanillin (Cava-Roda et al., 2012), conjugated linolenic acid (Lynch et al., 2005) and eugenol (Gaysinsky et al., 2007) have been tested for their potential to preserve milk. However, they have several limitations, including problems with solubility and stability, a tendency for oxidative degradation, the need for emulsifiers, the inability to use high-fat products such as milk, and limited antimicrobial efficacy (Shakeel et al., 2015; Badawy et al., 2023).

Phytochemicals such as catechin (Sekhavatizadeh et al., 2023), quercetin (De Souza et al., 2025), gallic acid (Sharma et al., 2019), sinapic acid (Rehim et al., 2023), betanin (Salman et al., 2024) and glycyrrhetinic acid (Amini et al., 2024) effectively preserve dairy products like cheese, soft cheese, and yoghurt without negatively impacting their sensory qualities. Although these compounds have primarily been studied in dairy products, they have not yet been tested in milk. This presents an opportunity to explore their potential as alternative methods for extending the shelf life and preserving milk. Therefore, the current study aims to evaluate the antioxidant and antimicrobial effects *in vitro* of these phytochemicals (catechin, quercetin, gallic acid, sinapic acid, betanin and glycyrrhetinic acid) and identify which compound is most effective against bacterial contamination and chemical oxidation in pasteurized milk.

3. Materials and Methods

3.1. Materials

The phytochemicals such as Catechin (C1251), Quercetin (Q4951), Gallic acid (G7384), Sinapic acid (D7927), Betanin (901266) and Glycyrrhetinic acid (G0149) are purchased from Sigma Aldrich. Each compound (50 mg) was dissolved in 250 µL Dimethyl sulfoxide (DMSO) followed by adding 750 µL of water (50 mg/mL) and used for further experiments (Mektrirat et al., 2023). Additionally, potassium dichromate (10 mg/mL), ampicillin (1 mg/mL) and ascorbic acid (10 mg/mL) were used in the study.

3.2. Antimicrobial activity

Escherichia coli (a Gram-negative bacterium, ATCC No. 43895 serotype O157:H7) and *Listeria monocytogenes* (a Gram-positive bacterium, ATCC No. 19111) were used as model organisms to assess the antibacterial property of phytochemicals. As both strains are classified under Biosafety Level 2 (BSL-2), all experimental works were conducted strictly in accordance with BSL-2 biosafety guidelines, using appropriate containment facilities and standard operating procedures approved by the Institutional Biosafety Committee (IBSC approval No.: SASTRA/IBSC/14/2023 dt. 08-11-2023). The agar well diffusion method was utilized to screen phytochemicals based on their zone of inhibition (ZOI) (Valgas et al., 2007). The minimum inhibitory concentration (MIC) of the phytochemicals was determined by measuring bacterial cell viability at a wavelength of 600 nm, using a BioTek Synergy H1 multimode microplate reader (Kiran et al., 2024). The structural integrity of the bacterial cells exposed to the selected active compound was evaluated using a VEGA3 TESCAN scanning electron microscope (SEM) at a magnification of 45,000x. This assessment followed the fixation of the samples with 2% glutaraldehyde, dehydration through a graded series of ethanol (25-100%), air drying, and gold coating (Tian et al., 2022a).

3.3. Antioxidant activity

The antioxidant activity of phytochemicals was investigated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method (Jankovic et al., 2023). A thiobarbituric acid reactive substances (TBARS) assay was used to analyze the in vitro inhibition capacity of selected active compounds against induced lipid peroxidation in milk (Bricarello et al., 2004). Additionally, an *in vitro* carbonyl assay was conducted to assess the inhibition potential of the selected active compounds against induced protein oxidation in milk (Milkovska-Stamenova et al., 2017).

3.4. Milk treatment

Fresh raw cow milk was collected from the cattle shed maintained by SASTRA Deemed University in Thanjavur, India. It was pasteurized at 65 °C for 20 minutes using an induction stove (Prestige PIC 1.0 V2) (Sfakianakis and Tzia, 2014). This method was chosen for its relevance in both industrial and domestic settings, as it effectively reduces the microbial load while preserving the nutritional and sensory qualities of milk. Pasteurized milk samples were handled aseptically in sterile containers and stored in a laboratory refrigeration, where the temperature was set and kept at 4 °C throughout the experiment. This standard storage condition was used to inhibit microbial growth, reinfection, slow down enzymatic and oxidative reactions, ensuring uniformity across treatments. Pasteurized milk was divided into four sterile containers treated with gallic acid (43 µg/mL), ascorbic acid (38 µg/mL), and potassium dichromate (122 µg/mL), and one untreated control. The concentrations were selected based on antioxidant IC_{50} and antimicrobial MIC values (Supplementary Figure 1). Ascorbic acid was included as a positive control due to its well-established antioxidant activity, enabling comparison with the effectiveness of gallic acid in limiting oxidative reactions during storage. All milk samples were stored at 4 °C and analysed for colony forming units (CFU) on days 0, 2, and 4 (Berhe et al., 2020). The concentrations of MDA and carbonyls in both control and treated milk were measured over a storage period of four days (Scheidegger et al., 2010; Oancea et al., 2022). The sensory quality of the milk treated with preservatives was evaluated on the 0th, 2nd, and 4th days using an untrained panel of 30 members (Tyl et al., 2024). Milk samples were evaluated based on appearance, color, and aroma characteristics using a 9-point scale, where a score of 9 signifies excellent quality, scores of 7 to 8 indicate good quality, scores of 5 to 6 represent fair quality, and scores below 5 reflect poor quality. The sensory qualities of color, aroma, and appearance were determined by calculating the average ratings of the individual panel members.

3.5. Statistical analysis

The results are presented in the form of mean ± SD. All experiments were carried out in triplicate except for SEM. The data obtained was analysed using one-way analysis of variance (ANOVA) followed by Tukey's test. Statistical analysis was performed using Graphpad Prism (Version 10).

4. Results and discussion

4.1. Zone of inhibition

The antibacterial profile of phytochemical compounds is ranked in the following order: Gallic acid > Catechin > Quercetin > Glycyrrhetinic acid = Sinapic acid = Betanin (Figure 1A). Among these phytochemicals, gallic acid demonstrated significantly higher antibacterial activity against the bacterial pathogen *E. coli,* with an inhibition zone of 18.67 mm, compared to the other phytochemicals, which had inhibition zones ranging from 9.33 to 11.33 mm. Ampicillin was included in the study as a positive control to serve as a benchmark for antimicrobial activity. Its inclusion allows for a comparative evaluation of the efficacy of the phytochemicals tested against *E. coli* and *L. monocytogenes*, providing a reference point with a well-established antibiotic to validate and contextualize the observed antibacterial effects. Previous reports have indicated that gallic acid has an antibacterial effectiveness against the foodborne pathogen *E. coli*, showing a zone of inhibition of 20 mm (Selvaraj et al., 2022). Additionally, Oliveira et al. (2019) studied the antimicrobial activity of gallic acid against *E. coli* O157:H7 (ATCC-700728) in apple juice, finding that gallic acid inhibits the activity of key enzymes involved in metabolism and cell wall synthesis.

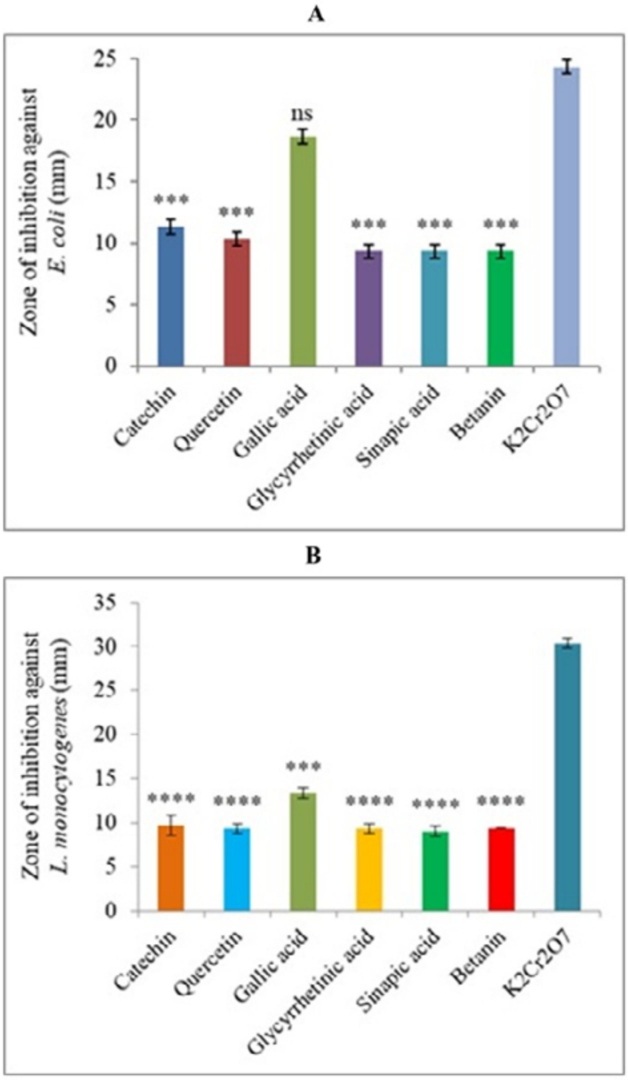


Figure 1. Zone of inhibition of phytochemicals against E. coli *(A) and* L. monocytogenes *(B). Mean values of each phytochemical were compared with the chemical preservative potassium dichromate ($K_2Cr_2O_7$) using ANOVA with Tukey's post-hoc test. The symbol '*' on the bars indicate the values are statistically significant (** $p < 0.01$, *** $p < 0.001$ & **** $p < 0.0001$) and 'ns' means not significant.*

Gallic acid displayed a notable antibacterial activity against Gram-positive (*L. monocytogenes*) bacteria with an inhibition zone of 13 mm (Figure 1B). According to Zamuz et al. (2021), gallic acid has the ability to compromise the structural integrity of the cell membrane in *L. monocytogenes.* Additionally, past studies indicate that gallic acid can be used as a preservative to inhibit *Yersinia enterocolitica* and extend the shelf life of pork at a concentration of 15 mg/g (Tian et al., 2022b). Furthermore, Sorrentino et al. (2018) reported that gallic acid helps preserve fresh black truffles from spoilage caused by *Pseudomonas* species during refrigeration storage. Gallic acid is considered edible and is generally recognized as safe for consumption, with the FAO stating that it is non-toxic at levels up to 5 g/kg of body weight when taken orally (Rajalakshmi et al., 2001). Niho et al. (2001) found that when administered orally, gallic acid is metabolized in the liver and excreted through the kidneys into the urine.

4.2. Minimum inhibitory concentration (MIC)

The MIC of the phytochemicals was represented in Figure 2A-B, where gallic acid exhibited the notably lowest MIC value of 3.12 mg/mL (18.3 mM) against *E. coli* and 6.25 mg/mL (36.7 mM) against *L. monocytogenes*, reflecting its superior antibacterial activity when compared to other phytochemicals, which showed MIC values of 12.5 mg/mL (ranging from 22.7 to 55.8 mM) as reported in Supplementary Table No. 1. However, potassium dichromate displayed a MIC of 0.39 mg/mL (1.33 mM) against *L. monocytogenes*, demonstrating greater potency than gallic acid. In agreement with our results, Diaz-Gomez et al. (2014) stated that MIC of 3.25 mg/mL (19.1 mM) of the gallic acid against *E. coli*. The study by Liu et al. (2022) emphasized that gallic acid strongly supresses *E. coli* through oxidative stress and cell death pathways, thus increasing ROS concentration, which triggers apoptotic markers and DNA fragmentation.

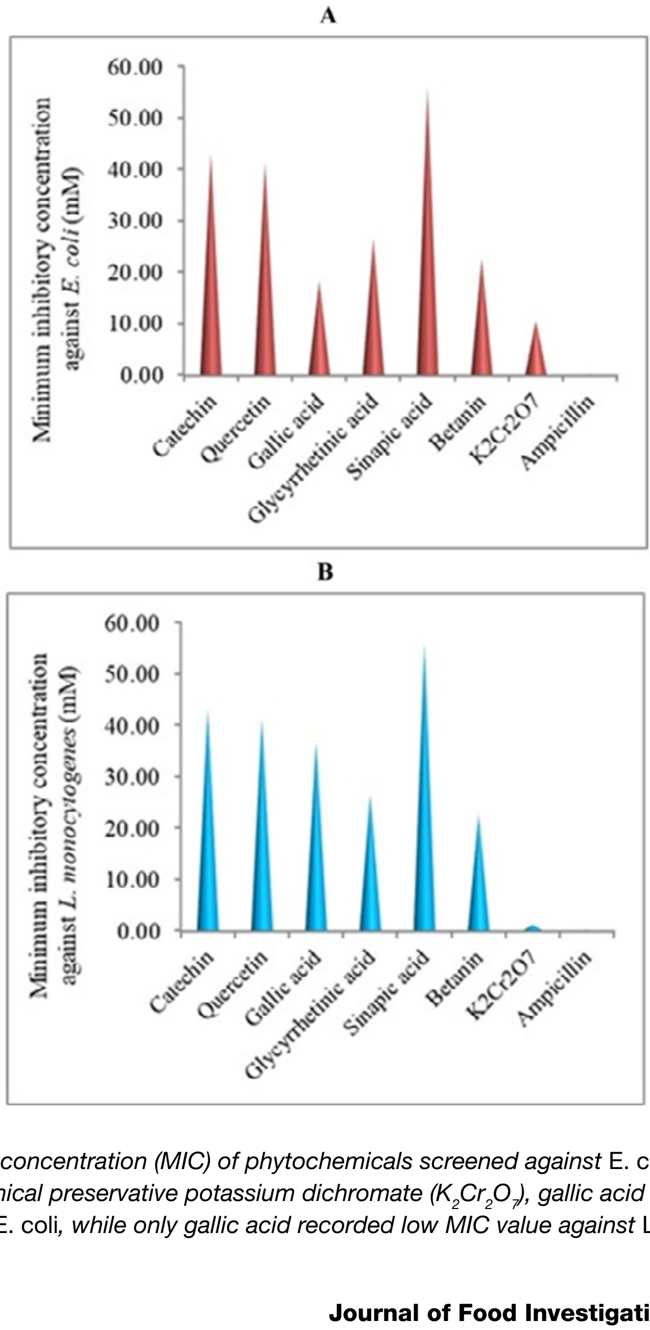


Figure 2 Minimum inhibitory concentration (MIC) of phytochemicals screened against E. coli *(A) and* L. monocytogenes *(B). In comparison with chemical preservative potassium dichromate ($K_2Cr_2O_7$), gallic acid was found to exhibit low MIC values in case of* E. coli*, while only gallic acid recorded low MIC value against* L. monocytogenes*.*

4.3. SEM analysis

The effect of gallic acid on the membrane structure of *E. coli* and *L. monocytogenes* was examined using scanning electron microscopy (SEM) at a magnification of 45,000 times, as shown in Figures 3A and 3B. In the control group, both *E. coli* and *L. monocytogenes* exhibited typical membrane morphology. However, noticeable changes in the cell membrane were observed after treatment with the minimum inhibitory concentration (MIC) of gallic acid, which was 3.125 mg/mL for *E. coli* and 6.25 mg/mL for *L. monocytogenes*. The SEM images revealed significant disruption and leakage of cellular contents in cells treated with gallic acid. These findings highlight the substantial impact of gallic acid on foodborne pathogenic bacteria, leading to alterations in cell morphology and membrane integrity. Consistent with our results, previous studies have reported that gallic acid causes morphological changes and damages the outer and inner membranes of *E. coli* (Tian et al., 2022a). The partial hydrophobicity of gallic acid, along with its ability to chelate divalent cations, was identified as a key factor in its function as a potent destabilizer of the membrane. Consequently, this destabilization increases membrane fluidity, leads to leakage of cellular contents, and ultimately compromises the integrity of bacterial cells. In the case of *L. monocytogenes*, the antimicrobial activity of gallic acid stems from its ability to disrupt bacterial cell membranes, compromising cell integrity and increasing membrane permeability (Muthulakshmi et al., 2022).

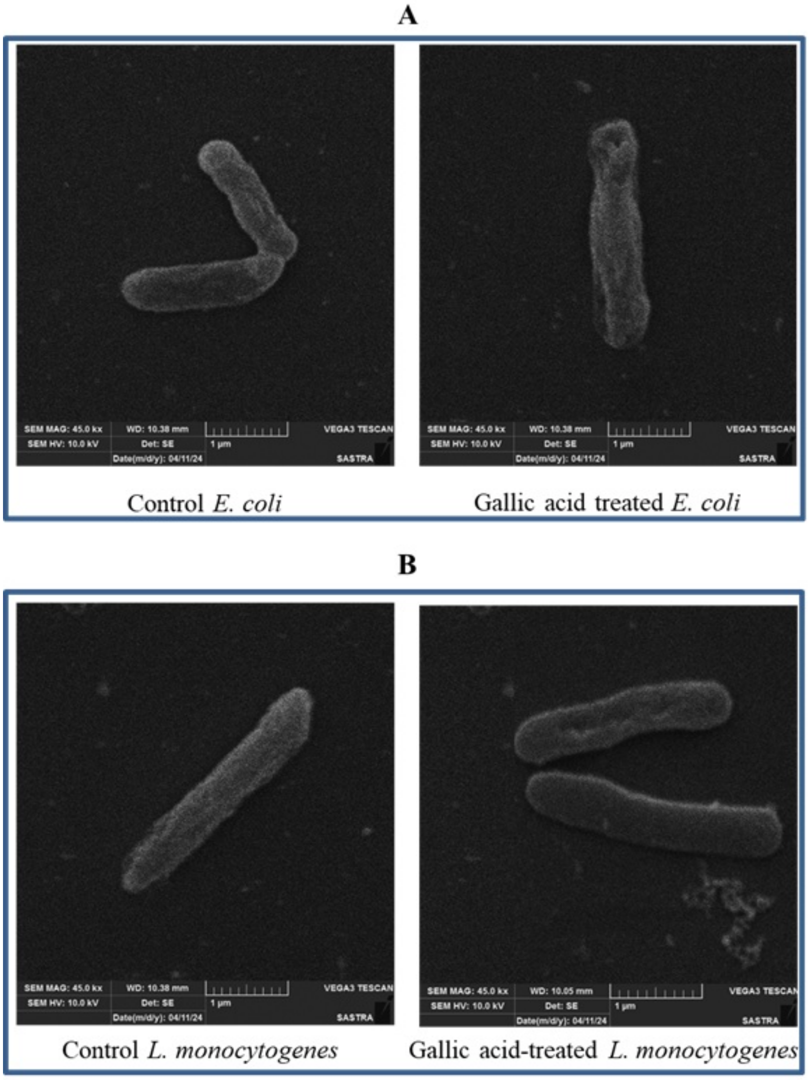


Figure 3. SEM analysis of E. coli *(A) and* L. monocytogenes *(B) with and without treatment of gallic acid. In gallic acid-treated cells, changes in the surface morphology of membrane in* E. coli *and* L. monocytogenes *were visualized while the cell membrane of control remains intact.*

4.4. In vitro antioxidant activity

Compared to potassium dichromate (44.05%), all the phytochemicals demonstrated significantly higher levels of antioxidant activity (ranging from 79.6% to 98.8%) with a statistical significance of $p < 0.05$, except for glycyrrhetinic acid, which showed an activity level of 66.66% (see Figure 4). Among the phytochemicals tested, quercetin, catechin, and gallic acid exhibited low IC50 values of 29, 40, and 43 µg/mL, respectively,

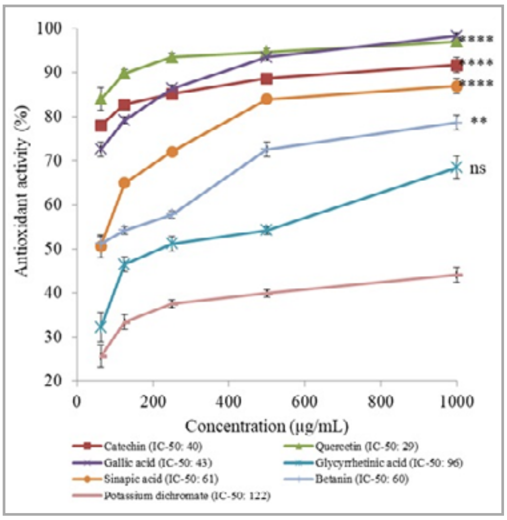


Figure 4. The antioxidant activity of different concentrations of selected phytochemicals against DPPH radicals. Mean values of each phytochemical were compared with the chemical preservative potassium dichromate ($K_2Cr_2O_7$) using ANOVA with Tukey's post-hoc test. The symbol '' on the bars indicate the values are statistically significant (** $p < 0.01$ & **** $p < 0.0001$) and 'ns' means not significant.*

which indicates their strong antioxidant activity in scavenging DPPH radicals. Although quercetin and catechin showed superior antioxidant activity compared to gallic acid, they were not selected for further experiments in this study due to their minimal antibacterial effect. In alignment with our research objective, we chose to use gallic acid for subsequent milk treatments. Gallic acid, which contains an additional carboxyl group, enhances its ability to donate hydrogen atoms, neutralizing free radicals, and preventing oxidative damage (Rafiee et al., 2018). On the contrary, the strong oxidizing nature of potassium dichromate can lead to the oxidation of various components of milk, such as lipids and proteins (Vaitkus and Kazlauskaite, 1977). This oxidation process may generate off-flavors, rancidity, and degradation of nutritional components, ultimately leading to the spoilage of milk. Therefore, using potassium dichromate as a preservative in milk would not only fail to prevent spoilage, but could also accelerate deterioration due to its oxidative effects. It is crucial to avoid using strong oxidizing agents in food products.

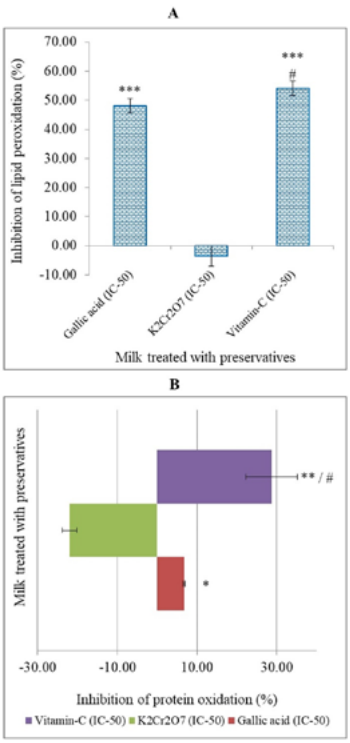


Figure 5. Inhibition capacity of gallic acid against induced lipid peroxidation (A) and protein oxidation (B). Mean values of gallic acid and Ascorbic acid were compared with the chemical preservative potassium dichromate ($K_2Cr_2O_7$) using ANOVA with Tukey's post-hoc test. The symbol '' on the bars indicate the values of gallic acid and Ascorbic acid are statistically significant (* $p < 0.05$, ** $p < 0.01$ & **** $p < 0.0001$) when compared to $K_2Cr_2O_7$ treated sample. The symbol '#" indicates the value of Ascorbic acid is significantly different when compared to gallic acid (#$p < 0.05$) and 'ns' means not significant.*

4.5. Inhibition of induced lipid peroxidation

Gallic acid exhibited a significant inhibition of induced lipid peroxidation in milk, showing a reduction of 48.21% ($p < 0.001$) compared to potassium dichromate (Figure 5A). Its capacity to inhibit lipid peroxidation was comparable to that of ascorbic acid, which achieved a 54.10% reduction. Supporting our findings, Farhoosh and Nyström (2018) reported that gallic acid inhibits lipid peroxidation in sunflower oil. Additionally, gallic acid has been utilized in coating materials for pork due to its effective prevention of lipid oxidation (Cao et al., 2019). Conversely, potassium dichromate, a strong oxidizing agent, increased lipid peroxidation, resulting in a negative inhibition percentage of -5.9%, indicating its pro-oxidant effect when applied to milk (Sahu et al., 2001). This negative impact highlights the unsuitability of potassium dichromateas a preservative in food products.

4.6. Inhibition of induced protein oxidation

In comparison to potassium dichromate, gallic acid-treated milk showed a significant reduction ($p < 0.05$) in induced protein oxidation, approximately 6.78% (see Figure 5B). These findings demonstrate the effectiveness of gallic acid in inhibiting protein oxidation in milk and reducing the formation of carbonyl compounds. Proteins are vulnerable to oxidation by reactive oxygen species (ROS) such as hydroxyl radicals, superoxide, and hydrogen peroxide. This interaction leads to the formation of protein radicals and subsequent oxidative modifications (Hellwig, 2020). Additionally, ROS can generate secondary reactive intermediates, including lipid peroxides and reactive aldehydes, which can further oxidize proteins (Kehm et al., 2021). Given the free radical scavenging ability observed in this study (see Figure 4), gallic acid can be utilized as a preservative to inhibit protein oxidation in milk, thus helping to prevent spoilage. In contrast, potassium dichromate, a strong oxidizing agent, increased protein oxidation and led to the formation of carbonyl compounds with a negative inhibition percentage of -22.01%. Supporting our results, Jaradat (2023) stated that exposure to hexavalent chromium of potassium dichromate induces protein oxidation in erythrocytes by ROS.

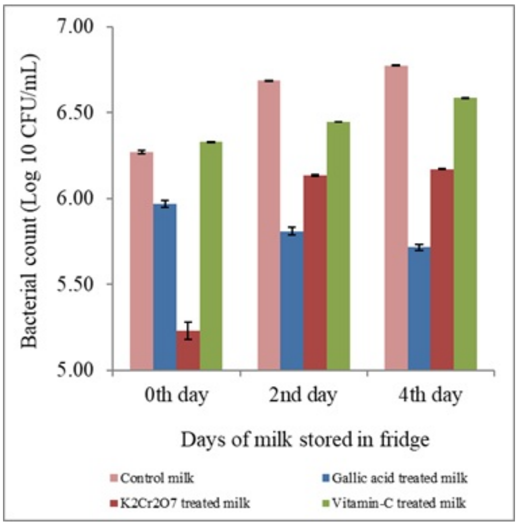


Figure 6. Total bacterial count of untreated control, gallic acid-treated, $K_2Cr_2O_7$-treated and Ascorbic acid-treated milk at 0, 2 & 4th day of storage at 4°C.

4.7. Microbial count of milk

The total bacterial count in untreated pasteurized milk (control) showed a substantial increase in colony count from 6.26 log_{10} CFU/mL on Day 0 to 6.77 log_{10} CFU/mL on day 4, indicating unrestricted bacterial growth (Figure 6 & Supplementary Figure 2). For contextual comparison, raw milk showed a comparatively higher initial microbial load of 6.71 log10 CFU/mL on day 0 (data not shown), representing a potential food safety concern for the possible presence of pathogenic microorganisms. According to FAO regulatory standards, raw milk with bacterial counts below 6.77 log_{10} CFU/mL is considered microbiologically safe for human consumption. The raw milk used in this study exceeded this threshold, highlighting the crucial role and effectiveness of pasteurization in substantially lowering microbial populations. Pasteurization achieves this reduction by applying thermal treatment, which inactivates bacterial cells and slows down enzymatic activities, thereby enhancing the safety of milk. The marked difference between raw and pasteurized milk highlights the importance of pasteurization in reducing the overall microbial load and improving the hygienic quality. In agreement with our results, Lim et al., (2023) reported a mean bacterial count of 6.24 log10 CFU/mL while Mengstu et al., (2023) found a microbial count 6.19 log10 CFU/mL in pasteurized milk. The pasteurized milk sample treated with gallic acid showed lower CFU count even on day 0 when compared to the untreated control. This difference might be due to the execution of the microbial count experiment performed 4 hours after the addition of gallic acid to pasteurized milk, which provides adequate time for the degradation of the microbiota by the preservative in milk. As a result, plates for different treatments show varying colony numbers and types, even on day 0 (Supplementary Figure 2). The colony counts in gallic acid treated milk decreased from 5.96 log10 CFU/mL on day 0 to 5.80 log10 CFU/mL on day 2, and further reduced to 5.71 log10 CFU/mL on day 4. This trend suggested that gallic acid exerts a suppressive effect on bacterial proliferation in milk, leading to a decline in bacterial populations over time. However, since these observations are based solely on total viable counts, they do not provide evidence for the reduction or elimination of specific pathogenic microorganisms, confirmation of pathogen-specific effects would require selective and differential microbiological analyses, which are need to be carried out in future investigation. Consistent with our results, Sorrentino et al. (2018) observed that gallic acid treatment in other foods such as truffles significantly reduced undesirable microorganisms (5.49 log10 CFU/mL) compared to the control (8.70 log10CFU/mL). Similarly, Zheng et al. (2018) reported that meat treated with gallic acid-grafted chitosan had a lower growth of coliform bacteria (3.08 log10 CFU/mL) than the control (3.45 log10 CFU/mL).

In this project, potassium dichromate initially demonstrated a reduction in bacterial colonies in day 2 (6.13 log10 CFU/mL) and day 4 (6.17 log10 CFU/mL) when compared with day 0 (6.22 log10 CFU/mL) in pasteurized milk. This suggests that potassium dichromate controlled the microbial population in pasteurized milk during the storage period, but was not superior compared to that of gallic acid. Additionally, due to pro-oxidant effects, potassium dichromate accelerates the oxidation of lipids and proteins in milk (it was observed under MDA and carbonyl tests in this project), and it is unsuitable as a preservative in milk. On the contrary, ascorbic acid treated milk exhibited an increase in bacterial colonies throughout the four-day period (from 6.32 log10 CFU/mL on day 0 to 6.58 log10 CFU/mL on day 4), highlighting that ascorbic acid treatments fail to inhibit bacterial proliferation. Although ascorbic acid provides a notable antioxidant benefit (noted in MDA and carbonyl tests of this project) and helps prevent oxidative damage in milk, it showed no antibacterial activity, which highlighting its ineffectiveness in controlling microbial growth in milk. Bacterial contamination can lead to spoilage, altering the taste, texture and odour of the milk, making it unsuitable for consumption. The uncontrolled growth of bacteria in milk poses significant health risks to consumers and the implications of consuming spoilt food extend beyond mere dissatisfaction with taste and texture. Consumption of spoilt milk contaminated with harmful bacteria such as *E. coli, Salmonella spp.*, and *L. monocytogenes* can cause severe health issues, including foodborne illnesses and gastrointestinal infections (Vahedi et al., 2013). Treatment with gallic acid can mitigate health risks through its antioxidant and antimicrobial properties, thus maintaining the safety of milk. However, the differences observed among the treatments in this study were not statistically significant. Small variations in bacterial counts may not fully reflect the true effect of the treatments, and extending the duration of treatment could potentially result in statistically significant reductions.

4.8. MDA concentration in milk

The MDA concentration in milk treated with preservatives was analyzed over four consecutive days, and the results are presented in Figure 7A. On the fourth day of storage, milk treated with potassium dichromate exhibited a significantly higher MDA concentration (21.51 mM) compared to several other groups: pasteurized control milk (18.60 mM), gallic acid-treated milk (10.46 mM), and ascorbic acid-treated milk (8.702 mM), with a significance level of $p < 0.05$. This suggests that potassium dichromate may induce oxidative stress or contribute to lipid peroxidation in milk, resulting in elevated MDA levels. Inclusion of potassium dichromate as a preservative in milk leads to the generation of hydroxyl radicals (Brown and Thurston, 1940). Reactive radicals play a significant role in the increase in lipid peroxidation. They can remove hydrogen atoms from the polyunsaturated fatty acids found in milk, triggering lipid peroxidation, a chain reaction that ultimately destroys lipids and produces toxic by-products. Absorption of these oxidized lipids can initiate an inflammatory response that affects various organs, including the liver, kidneys, lungs, and gut (Kanner, 2007). Regular consumption of oxidized fats poses a continuous risk to human health, potentially leading to chronic health issues (Grootveld et al., 2018). Research shows that milk treated with gallic acid has a low concentration of malondialdehyde (MDA), similar to that of milk treated with ascorbic acid. This is attributed to their strong antioxidant properties. The ability of gallic acid to scavenge reactive oxygen species (ROS) and its antioxidant qualities can impede oxidation processes when it is incorporated into food. Supporting our findings, Huang et al. (2021) reported that a gallic acid-pectin coating significantly reduced MDA levels to 0.21 mg/kg compared to 0.85 mg/kg in the control group after five days of storage, effectively preserving the quality of Chinese sea bass fillets during refrigeration. Similarly, Fang et al. (2018) found a reduction in lipid oxidation in gallic acid-coated pork samples, with MDA levels at 0.252 mg/kg compared to 1.004 mg/kg in the control group after five days of storage.

4.9. Protein carbonyl concentration in milk

The effect of different preservatives on protein carbonyl concentration in both pasteurized control milk and gallic acid treated milk was analyzed over a period of four days, with the results presented in Figure 7B. Compared to the control group, which had a protein carbonyl concentration of 20.00 nM, the concentrations for ascorbic acid (14.77 nM) and gallic acid (16.59 nM) were lower. However, milk treated with potassium dichromate exhibited an increase in protein carbonyl concentration, reaching 22.05 nM on day 4. This finding indicates that potassium dichromate promotes protein oxidation, leading to an increase in protein carbonyl formation in milk, which may compromise its quality and nutritional value. Reactive oxygen species (ROS) can trigger the oxidation of milk proteins, particularly affecting methionine residues due to the presence of sulphur-containing groups. When methionine is oxidized, it transforms into sulfenic acid (-SOH), which can then interact with nearby methionine residues, resulting in the formation of disulphide bonds within or between proteins. Although these bonds are essential for maintaining protein structure and stability, excessive or inappropriate formation of disulphide bonds can lead to protein aggregation. This disruption in protein-protein interactions can impair various physiological and biochemical processes in milk and dairy products (Wüst and Pischetsrieder, 2016).

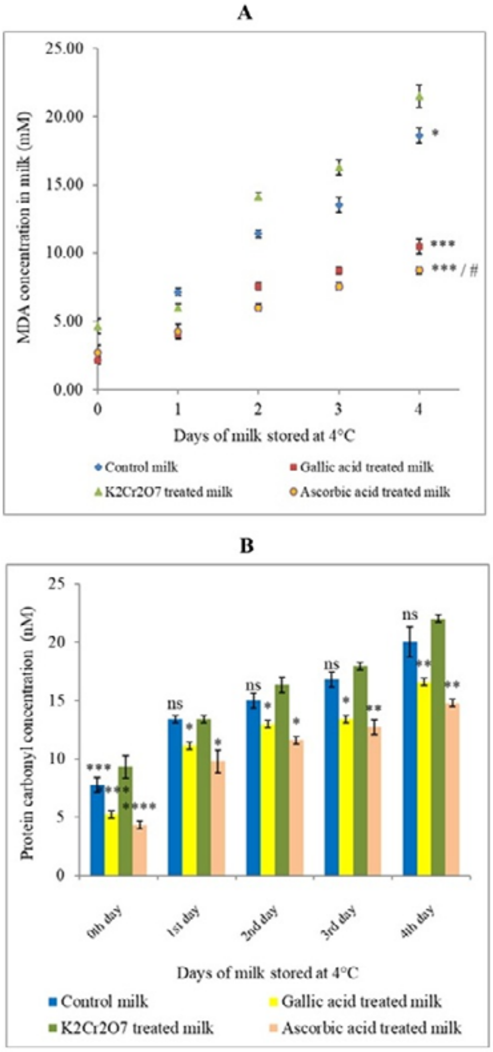


Figure 7. MDA concentration (A) and protein carbonyl concentration (B) of untreated control, gallic acid-treated, $K_2Cr_2O_7$-treated and Ascorbic acid -treated milk at 0, 2 & 4th day of storage at 4°C. Mean values of each treatment were compared with $K_2Cr_2O_7$-treated milk using ANOVA with Tukey's post-hoc test. The symbol '' on the last day of storage (4th day) indicate the values of gallic acid and Ascorbic acid are statistically significant (* $p < 0.05$ & *** $p < 0.001$) when compared to $K_2Cr_2O_7$ treated sample. The symbol '#" indicates the value of Ascorbic acid is significantly different when compared to gallic acid (# $p < 0.05$).*

Unlike potassium dichromate, samples treated with gallic acid (1.32-fold) and ascorbic acid (1.49-fold) demonstrated a significant reduction ($p < 0.05$) in the formation of carbonyl compounds in milk. The carbonyl content in gallic acid-treated milk was found to be similar to that of ascorbic acid-treated milk, with only a 10.97% difference between the two. Supporting our findings, Adisakwattana et al. (2017) observed that gallic acid lowered protein carbonyl levels, measuring 0.64 nM/mg of protein, compared to the control, which had 1.23 nM/mg protein, in an in vitro food model. Similarly, Fang et al. (2018) reported that pork coated with gallic acid exhibited a significantly lower carbonyl value of 64.80 nM/mg protein after five days of storage, compared to the control value of 66.31 nM/mg protein. Gallic acid has been used as a nucleophilic scavenger to target carbonyl compounds such as glyoxal and methylglyoxal. This compound effectively intercepts and neutralizes these toxic substances, which contribute to harmful carbonyl species in e-cigarette aerosols (Falco et al., 2020). Additionally, carbonylated proteins, which form during food processing and storage, can undergo structural changes that impair digestibility and reduce the nutritional value of food products. Consumption of these carbonylated proteins is associated with oxidative stress, inflammation, and age-related diseases (Estévez et al., 2022). This highlights the importance of preventing protein oxidation in milk through the use of gallic acid.

4.10. Sensory characteristics of milk

The sensory characteristics of pasteurized milk (untreated control, gallic acid-treated, ascorbic acid and potassium dichromate-treated) were presented in Figure 8A-C. On the 4th day of storage, the control milk showed evident signs of spoilage, such as a thicker texture and a rancid odour, when compared to days 0 and 2 (Supplementary Figure 1). Sensory evaluation of untreated milk on day 4 revealed poor quality, with scores of 3 for appearance, 2.7 for aroma, and 3.8 for color. Even on day 2, the aroma score decreased to 6.7 compared to day 0, when the control milk had a score of 10. The casein protein in untreated milk is vulnerable to denaturation due to changes in pH and enzymatic activity caused by spoilage microorganisms, leading to structural alterations and coagulation during storage (Sinaga et al., 2017). On the contrary, the milk sample treated with gallic acid maintained its natural characteristics throughout the storage period. On day 4, the appearance, aroma, and color scores were consistently high at 9, 8.5, and 9, respectively, indicating good quality in these attributes. This positive result is attributed to the antioxidant and antimicrobial properties of gallic acid. Chemical oxidation and microbial growth are the primary causes of milk quality deterioration, which leads to off-flavors, color changes, and nutrient degradation. Gallic acid, known for its antimicrobial and free radical scavenging properties, can prevent both oxidation reactions and microbial infections in milk, thus extending its shelf life and preserving its natural characteristics. Our findings align with previous research indicating that the incorporation of gallic acid into a gelatin-chitosan-glycerol coating improves the preservation of color (score 6.3), odor (score 6.4), and texture (score 6.1) of beef samples after five days of storage (Zou et al., 2022). According to a report by The New Indian Express (2024), pasteurized milk from Aavin (the Government Dairy in Tamil Nadu, India) can typically last up to two days when stored at 4°C, but it spoils in less than four hours if not properly refrigerated. In contrast, milk treated with gallic acid maintains good sensory qualities for up to four days, offering a significantly longer shelf life compared to untreated control milk.

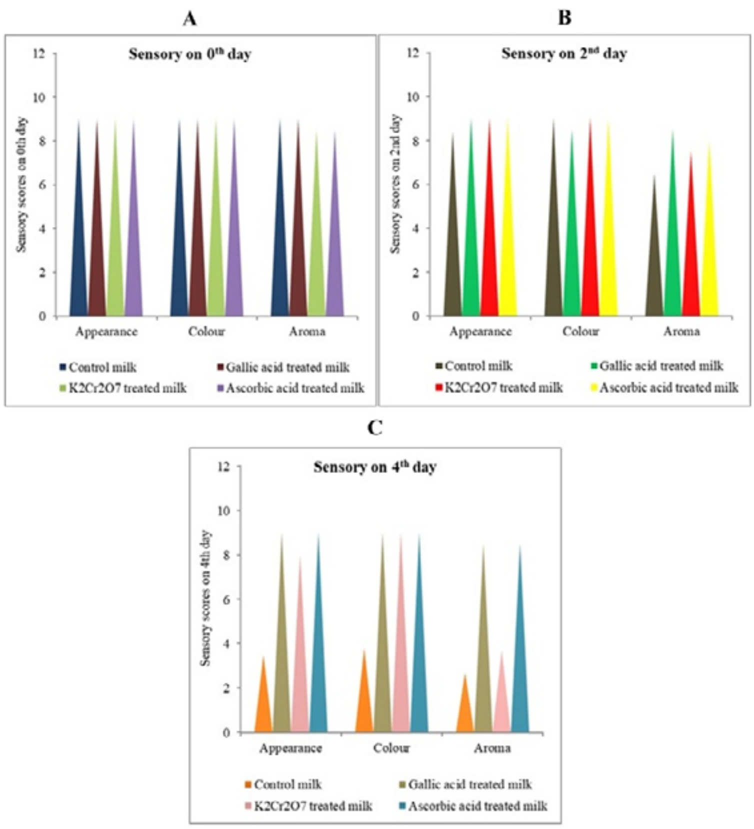


Figure 8. Sensory evaluation scores of control, gallic acid-treated, $K_2Cr_2O_7$-treated and Ascorbic acid -treated milk at 0th day (A), 2nd day (B) & 4th day (C). The aroma was found to be declined in both control and $K_2Cr_2O_7$-treated milk on 2nd day while all the sensory parameters were decreased in control on 4th day. The aroma in $K_2Cr_2O_7$-treated milk was un-acceptable on 4th day due to oxidative damages of lipids and formation of peroxides in milk.

Milk treated with potassium dichromate over four days showed a significant decline in aroma value (Figure 8A-C), indicating rancidity caused by lipid peroxidation and carbonyl formation, as recorded in the MDA and protein carbonyl tests conducted in this project. The oxidation reactions initiated by potassium dichromate trigger the oxidation of lipids and proteins in milk, leading to the formation of intermediate compounds such as MDA and propanal, which contribute to the progression of rancidity. These compounds produce off-flavors and degrade the quality of milk (Durham et al., 1978). During the experiment, the aroma value of potassium dichromate treated milk started at a rating of 10 on day 0 and declined to 7.8 on day 2, reflecting increased lipid peroxidation and the accumulation of carbonyl compounds in the milk. However, the color and appearance scores remained unaffected, both rated at 9. This discrepancy may arise because oxidation of milk primarily impacts flavor and aroma, while color and texture may not be noticeably influenced. Furthermore, the duration of treatment may not have been sufficient to detect changes in color or texture attributes. In milk treated with ascorbic acid, no significant changes in colour (score 9) and appearance (score 9) were observed. However, a strong pungent odour developed on the fourth day of storage in ascorbic acid treated milk, which reduced the sensory score to 5.1. The low pH provided by ascorbic acid could alter the bacterial biota in the milk, which metabolizes lactose and milk components, produces organic acids and volatile sulphur compounds that can impart a pungent smell to milk (Dordevic et al., 2023). In line with our findings, Sobczak et al. (2022) observed that the addition of ascorbic acid negatively affected the cow's milk yoghurt.

5. Conclusions

In the search for natural food preservatives, gallic acid has been identified as an effective antibacterial and antioxidant compound, outperforming other phytochemicals such as catechin, quercetin, sinapic acid, betanin, and glycyrrhetinic acid. Gallic acid demonstrated significant antibacterial activity against *E. coli* and *L. monocytogenes*, both of which are the main food pathogens found in milk. Scanning electron microscope (SEM) observations showed distinct morphological changes in bacterial cells, indicating alterations in the integrity of the cell membrane that were associated with a notable reduction in bacterial counts in milk. Furthermore, gallic acid effectively inhibited protein and lipid peroxidation in milk due to its strong antioxidant properties. Milk treated with gallic acid maintained good sensory qualities for up to four days, providing an extended shelf life compared to both the control group and milk treated with potassium dichromate during storage. This research shows that gallic acid is more efficient than traditional chemical preservatives such as potassium dichromate, suggesting its potential as a safe and environmentally friendly preservative for milk, pending further long-term storage studies at pilot-scale.

Supplementary file: The Supplementary file contains Supplementary Figures 1 & 2 and Supplementary Table 1.

Funding statement: This research work was not supported by any governmental / non-Governmental / public / private funding agencies.

Conflicts of interest/Competing interests: The authors declare that they have no conflict of interest.

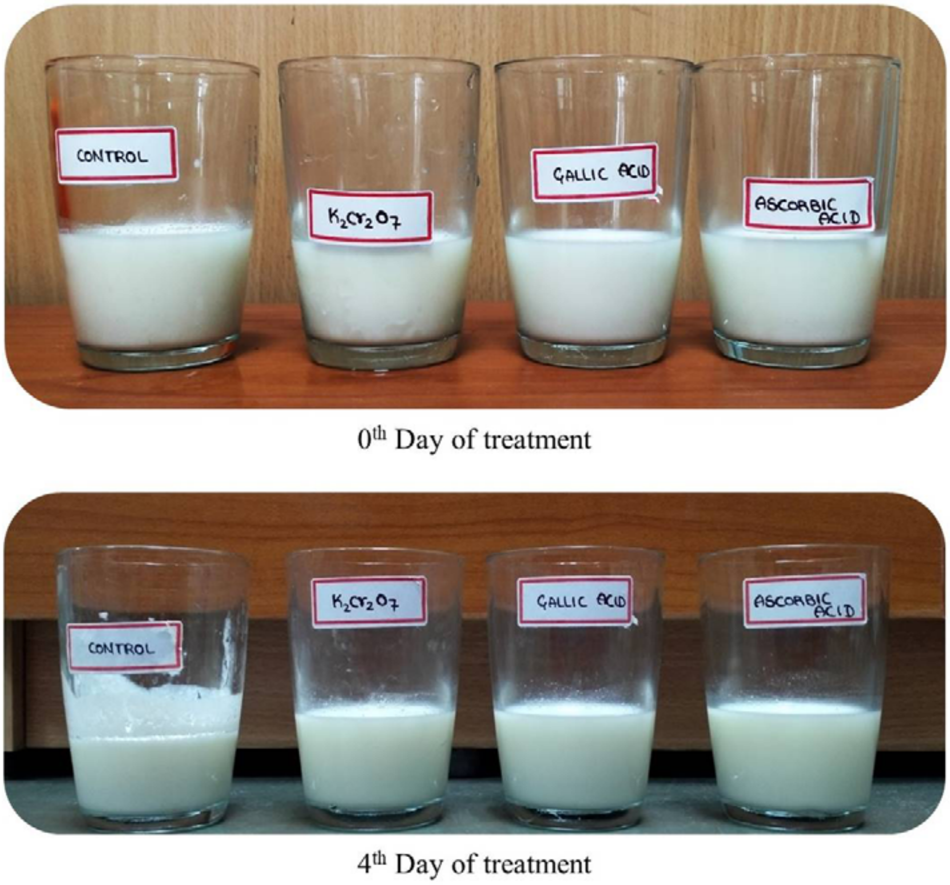
Ethical statement: There are no animal studies involved in the reported work. Institutional Bio-Safety Committee approval was obtained for experiments involving harmful pathogenic bacterial species (IBSC approval No.: SASTRA/IBSC/14/2023 dt. 08-11-2023). In sensory evaluation, appropriate protocols were applied for protecting the rights and privacy of all human participants, who are involved in sensory analysis. Full disclosure of the study requirements and risks was done with the verbal consent of the participants, and data of the participants is not released without their knowledge.

Consent for publication: All authors have given consent to publish the paper.

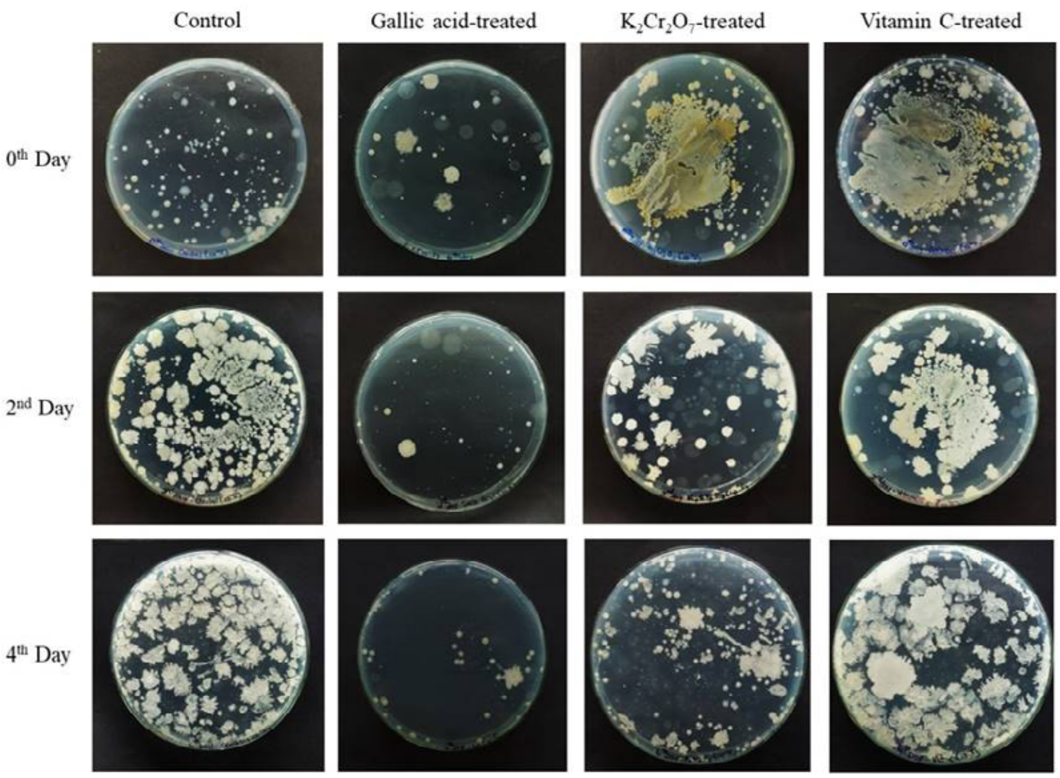
Availability of data and material: Data will be made available upon request.

Authors' contributions: All authors contributed to the design and implementation of the research, to the analysis of the results, and to the writing of the manuscript.

Acknowledgements: Authors are thankful to the Management and Administrative authorities of SASTRA Deemed University, Thanjavur, Tamilnadu for their encouragement and support.



Supplementary Figure 1.
Appearance of control, $K_2Cr_2O_7$-treated, gallic acid-treated and ascorbic acid-treated milk at 0th, 2nd & 4th day of storage at 4°C.



Supplementary Figure 2.
The total bacterial count visualized on nutrient agar plates for untreated control, gallic acid-treated, $K_2Cr_2O_7$-treated and vitamin-C-treated milk at 0th, 2nd & 4th day of refrigerated storage.

Supplementary Table 1.
Minimum Inhibitory Concentration (MIC) of tested compounds against E. coli and L. monocytogenes

S. No.	Phytochemicals	*E. coli*		*L. monocytogenes*	
		MIC (mg/mL)	MIC (mM)	MIC (mg/mL)	MIC (mM)
1	Catechin	12.50	43.06	12.50	43.06
2	Quercetin	12.50	41.36	12.50	41.36
3	Gallic acid	3.13	18.37	6.25	36.74
4	Glycyrrhetinic acid	12.50	26.56	12.50	26.56
5	Sinapic acid	12.50	55.75	12.50	55.75
6	Betanin	12.50	22.71	12.50	22.71
7	Potassium dichromate	3.13	10.62	0.39	1.33
8	Ampicillin	0.09	0.26	0.09	0.26

References

Abdel-Aziz MED, Darwish MS, Mohamed AH, El-Khateeb AY, Hamed SE. (2020). Potential activity of aqueous fig leaves extract, olive leaves extract and their mixture as natural preservatives to extend the shelf life of pasteurized buffalo milk. Foods, 9 (5): 615. DOI: 10.3390/foods9050615.

Adisakwattana S, Thilavech T, Sompong W, Pasukamonset P. (2017). Interaction between ascorbic acid and gallic acid in a model of fructose-mediated protein glycation and oxidation, Electronic Journal of Biotechnology, 27: 32-36, DOI: 10.1016/j.ejbt.2017.03.004.

Amini A, Mousavi ZE, Mousavi SM. (2024). Biological transformation of herbal extracts through lactic acid bacteria fermentation: A study on milk thistle and liquorice root extract. Food Bioscience, 62, 105105. DOI: 10.1016/j.fbio.2024.105105.

Badawy S, Liu Y, Guo M, Liu Z, Xie C, Marawan MA, Ares I, Lopez-Torres B, Martínez M, Maximiliano JE, Martínez-Larrañaga MR, Wang X, Anadón A, Martínez MA. (2023) Conjugated linoleic acid (CLA) as a functional food: Is it beneficial or not? Food Research International, 172: 113158. DOI: 10.1016/j.foodres.2023.113158.

BAHS, 2022. Available online at: https://dahd.nic.in/sites/default/filess/BAHS2023.pdf, downloaded on 22-03-2024.

Berhe G, Wasihun AG, Kassaye E. (2020). Milk-borne bacterial health hazards in milk produced for commercial purpose in Tigray, Northern Ethiopia. BMC Public Health, 20, 894. DOI: 10.1186/s12889-020-09016-6.

Beya MM, Netzel ME, Sultanbawa Y, Smyth H, Hoffman LC. (2021). Plant-based phenolic molecules as natural preservatives in comminuted meats: A review. Antioxidants (Basel), 10 (2): 263. DOI: 10.3390/antiox10020263.

Bricarello L, Kasinski N, Bertolami M, Faludi A, Pinto L, Relvas W, Izar MCO, Ihara S, Tufik S, Fonseca F. (2004). Comparison between the effects of soy milk and non-fat cow milk on lipid profile and lipid peroxidation in patients with primary hypercholesterolemia, Nutrition, 20 (2), 204. DOI: 10.1016/j.nut.2003.10.005.

Brown WC, Thurston LM. (1940). A review of oxidation in milk and milk products as related to flavour. Journal of Dairy Science, 23 (7), 629-685. DOI: 10.3168/jds.s0022-0302(40)95555-2.

Cao Y, Warner RD, Fang Z. (2019). Effect of chitosan/nisin/gallic acid coating on preservation of pork loin in high oxygen modified atmosphere packaging. Food Control, 101: 9-16, DOI: 10.1016/j.foodcont.2019.02.013.

Cava-Roda RM, Taboada-Rodríguez A, Valverde-Franco MT. (2012). Antimicrobial activity of vanillin and mixtures with cinnamon and clove essential oils in controlling Listeria monocytogenes and Escherichia coli O157:H7 in milk. Food Bioprocess Technology, 5, 2120-2131. DOI: 10.1007/s11947-010-0484-4.

Coutinho NM, Silveira MR, Rocha RS, Moraes J, Ferreira MVS, Pimentel TC, Freitas MQ, Silva MC, Raices RSL, Ranadheera CS, Borges FO, Mathias SP, Fernandes FAN, Rodrigues S, Cruz AG. (2018). Cold plasma processing of milk and dairy products. Trends in Food Science & Technology. 74, 56-68.

Chobpattana W, Jeon, IJ, Smith JS, Loughin TM. (2002). Mechanisms of interaction between vanillin and milk proteins in model systems. Journal of Food Science, 67 (3), 973–977. DOI: 10.1111/j.1365-2621.2002.tb09438.x.

De Souza PG, Padilha JV, Zanzarini IS, de Oliveira AHG, Moure VR, Valdameri G, Toci AT, Mafra MR, Farias FO and Igarashi-Mafra, L. (2025). High-value extracts from pulp, seeds, and leaves of Grumixama: Antioxidant, low cytotoxic, and preservative effect in cream cheese. Food Control, 110780, DOI: 10.1016/j.foodcont.2024.110780.

Díaz-Gomez R, Toledo-Araya H, López-Solís R, Obreque-Slier E. (2014). Combined effect of gallic acid and catechin against Escherichia coli. LWT-Food Science and Technology, 59 (2), 896-900. DOI: 10.1016/j.lwt.2014.06.049.

Dordevic D, Capikova J, Dordevic S, Tremlová B, Gajdács M, Kushkevych I. (2023). Sulfur content in foods and beverages and its role in human and animal metabolism: A scoping review of recent studies, Heliyon, 9 (4), 240-840, DOI: 10.1016/j.heliyon.2023.e15452.

Durham JR, Bechtle RM, Oberlander M. (1978). Chlorinated quaternized hexamine and potassium as preservatives for milk samples. Journal of Dairy Science, 61 (12), 1696-1699. DOI: 10.3168/jds.S0022-0302(78)83789-8.

Estévez M, Díaz-Velasco S, Martínez R. (2022). Protein carbonylation in food and nutrition: A concise update. Amino Acids, 54 (4), 559-573.

Falco B, Petridis A, Paramasivan P, Troise AD, Scaloni A, Deeni Y, Stephens WE, Fiore A. (2020). Reducing toxic reactive carbonyl species in e-cigarette emissions: Testing a harm- reduction strategy based on dicarbonyl trapping. RSC Advances, 10 (36), 21535- 21544. DOI: 10.1039/d0ra02138e.

Fang Z, Lin D, Warner RD, Ha M. (2018). Effect of gallic acid/chitosan coating on fresh pork quality in modified atmosphere packaging. Food Chemistry, 260: 90-96, DOI: 10.1016/j.foodchem.2018.04.005.

FAO, 2022. Available online at: https://www.fao.org/3/cc3418en/cc3418en.pdf, downloaded on 15-02-2024.

Farhoosh R, Nyström L. (2018). Antioxidant potency of gallic acid, methyl gallate and their combinations in sunflower oil triacylglycerols at high temperature. Food Chemistry, 244: 29-35. DOI: 10.1016/j.foodchem.2017.10.025.

Gaysinsky S, Taylor TM, Davidson PM, Bruce BD, Weiss J. (2007) Antimicrobial efficacy of eugenol microemulsions in milk against Listeria monocytogenes and Escherichia coli O157:H7. Journal of Food Protection, 70 (11): 2631-2737. DOI: 10.4315/0362-028x-70.11.2631.

Grootveld M, Percival BC, Grootveld KL. (2018). Chronic non-communicable disease risks presented by lipid oxidation products in fried foods. Hepatobiliary Surgery and Nutrition, 7 (4), 305-312.

Gross AS, 2018. Available online at: https://www.theguardian.com/environment/2023/ jan/06/about-animals-farmed-investigating-modern-farming-around-the-orld?CMP= share_btn_url, downloaded on 05-02-2024.

Hanrahan L, McHugh N, Hennessy T, Moran B, Kearney R, Wallace M, Shalloo L. (2018). Factors associated with profitability in pasture-based systems of milk production. Journal of Dairy Science, 101 (6), 5474-5485. DOI: 10.3168/jds.2017-13223.

Hellwig M. (2020). Analysis of protein oxidation in food and feed products. Journal of Agricultural and Food Chemistry. 68 (46), 12870-12885. DOI: 10.1021/acs.jafc.0c00711.

Huang B, Zhang Z, Ding N, Wang B, Zhang G, Fei P. (2021). Investigation of the pectin grafting with gallic acid and propyl gallate and their antioxidant activities, antibacterial activities and fresh keeping performance. International Journal of Biological Macromolecules, 190: 343-350, DOI: 10.1016/j.ijbiomac.2021.08.219.

Janković N, Tadić J, Milović E, Marković Z, Jeremić S, Petronijević J, Joksimović N, Borović TT, Abbas Bukhari SN. (2023). Investigation of the radical scavenging potential of vanillin-based pyrido-dipyrimidines: experimental and in silico approach. RSC Advances, 13 (22), 15236-15242. DOI: 10.1039/d3ra02469e.

Jaradat A. (2023) Antioxidant gallic acid protects clotting factor proteins against hydrogen peroxide induced oxidative injury. Research Journal of Pharmacy and Technology, 16 (7): 3463-3467. DOI: 10.52711/0974-360X.2023.00572.

Kabiraz MP, Majumdar PR, Mahmud MMC, Bhowmik S, Ali A. (2023) Conventional and advanced detection techniques of foodborne pathogens: A comprehensive review. Heliyon, 9 (4): e15482. DOI: 10.1016/j.heliyon.2023.e15482.

Kanner.J. (2007). Dietary advanced lipid oxidation end products are risk factors to human health. Molecular Nutrition & Food Research, 51 (9), 1094-1101. DOI: 10.1002/mnfr.200600303.

Kapusta A, Kuczyńska B, Puppel K. (2018). Relationship between the degree of antioxidant protection and the level of malondialdehyde in high-performance Polish Holstein-Friesian cows in peak of lactation. PloS One, 13 (3), e0193512. DOI: 10.1371/journal.pone.0193512.

Kehm R, Baldensperger T, Raupbach J, Höhn A, (2021). Protein oxidation - Formation mechanisms, detection and relevance as biomarkers in human diseases. Redox Biology, 42, 101901. DOI: 10.1016/j.redox.2021.101901.

Kiran S, Tariq A, Iqbal S, Naseem Z, Siddique W, Jabeen S, Bashir R, Hussain A, Rahman M, Habib FE, Rauf W, Ali A, Sarwar Y, Jander G, Iqbal M. Punicalagin, a pomegranate polyphenol sensitizes the activity of antibiotics against three MDR pathogens of the Enterobacteriaceae. (2024). BMC Complementary Medicine and Therapies. 16;24(1):93. Doi: 10.1186/s12906-024-04376-7.

Kroger, M. (1985). Milk sample preservation. Journal of Dairy Science, 68 (3), 783-787. DOI: 10.3168/jds.s0022-0302(85)80889-4.

Lim SH, Chin NL, Sulaiman A, Tay CH, Wong TH. (2023). Microbiological, physicochemical and nutritional properties of fresh cow milk treated with industrial high-pressure processing (HPP) during storage. Foods, 12 (3): 592, DOI: 10.3390/foods12030592.

Liu L, Ma X, Bilal M. (2022). Toxicity and inhibition mechanism of gallic acid on physiology and fermentation performance of Escherichia coli. Bioresources and Bioprocessing, 9, 76. DOI: 10.1186/s40643-022-00564-w.

Lynch JM, Lock AL, Dwyer DA, Noorbakhsh R, Barbano DM, Bauman DE. (2005). Flavor and stability of pasteurized milk with elevated levels of conjugated linoleic acid and vaccenic acid. Journal of Dairy Science, 88 (2): 489-498. DOI: 10.3168/jds. S0022-0302(05)72711-9.

Machado SG, Baglinière F, Marchand S, Van Coillie E, Vanetti MCD, De Block J and Heyndrickx M (2017). The biodiversity of the microbiota producing heat-resistant enzymes responsible for spoilage in processed bovine milk and dairy products. Frontiers in Microbiology, 8, 302.

Mafe AN, Edo GI, Makia RS, Joshua OA, Akpoghelie PO, Gaaz TS, Jikah AN, Yousif E, Isoje EF, Igbuku UA, Ahmed DS, Essaghah AE, Umar H. (2024). A review on food spoilage mechanisms, food borne diseases and commercial aspects of food preservation and processing.Food Chemistry Advances. (5), 100852. DOI: 10.1016/j.focha.2024.100852.

Martín-Miguélez JM, Martín I, Peromingo B, Delgado J, Córdoba JJ. (2025). Pathogen and Spoilage Microorganisms in Meat and Dairy Analogues: Occurrence and Control Strategies. Foods.14(10):1819. https://doi.org/10.3390/foods14101819

Mektrirat R, Chuammitri P, Navathong D, Khumma T, Srithanasuwan A and Suriyasathaporn W. (2023). Exploring the potential immunomodulatory effects of gallic acid on milk phagocytes in bovine mastitis caused by Staphylococcus aureus. Frontiers in Veterinary Science, 10, 1255058.

Mengstu B, Tola A, Nahusenay H, Sisay T, Kovac J, Vipham J, Zewdu A. (2023). Evaluation of microbial hygiene indicators in raw milk, pasteurised milk and cottage cheese collected across the dairy value chain in Ethiopia. International Dairy Journal, 136: 105487, DOI: 10.1016/j.idairyj.2022.105487.

Milkovska-Stamenova S, Mnatsakanyan R, Hoffmann R. (2017). Protein carbonylation sites in bovine raw milk and processed milk products. Chemistry, 229, 417-424. DOI: 10.1016/j.foodchem.2017.02.102.

Muthulakshmi L, Suganya K, Murugan M, Annaraj J, Duraipandiyan V, Farraj D, Elshikh M, Juliet A, Pasupuleti M, Arockiaraj J. (2022). Antibiofilm efficacy of novel biogenic silver nanoparticles from Terminalia catappa against food-borne Listeria monocytogenes ATCC 15,313 and mechanisms investigation in-vivo and in-vitro. Journal of King Saud University-Science, 34 (5) 102083, DOI: 10.1016/j.jksus.2022.102083.

Niho N, Shibutani M, Tamura T, Toyoda K, Uneyama C, Takahashi N, Hirose M (2001). Subchronic toxicity study of gallic acid by oral administration in F344 rats. Food and Chemical Toxicology, 39 (11), 1063-1070.

Oancea AG, Untea AE, Dragomir C, Radu GL. (2022). Determination of optimum TBARS conditions for evaluation of cow and sheep milk oxidative stability. Applied Sciences, 12 (13): 6508. DOI: 10.3390/app12136508.

Oliveira E, Nguyen C, Stepanian K, Cossu A, Nitin N. (2019). Enhanced bacterial inactivation in apple juice by synergistic interactions between phenolic acids and mild food processing technologies. Innovative Food Science & Emerging Technologies, 56, 102186. DOI: 10.1016/j.ifset.2019.102186.

Pereira PC. (2014). Milk nutritional composition and its role in human health. Nutrition, 30 (6): 619-627. DOI: 10.1016/j.nut.2013.10.011.

Rafiee SA, Farhoosh R, Sharif A. (2018). Antioxidant activity of gallic acid as affected by an extra carboxyl group than pyrogallol in various oxidative environments. European Journal of Lipid Science and Technology, 120 (11): 1800319. DOI: 10.1002/ejlt.201800319.

Rajalakshmi K, Devaraj H, Niranjali Devaraj S. (2001). Assessment of the no-observed- adverse-effect level (NOAEL) of gallic acid in mice. Food and Chemical Toxicology, 39 (9), 919-922. DOI: 10.1016/s0278-6915(01)00022-9.

Rehim MA, Zahran HA, Moghazy MA. (2023). Synthesis of active packaging films from Lepidium sativum gum/polyvinyl alcohol composites and their application in preserving cheddar cheese. Scientific Reports, 13, 1647. DOI:10.1038/s41598-023-28173-3.

Saha S, Majumder R, Rout P, Hossain S. (2024). Unveiling the significance of psychotropic bacteria in milk and milk product spoilage – A review, The Microbe, 2, 100034,

Sahu SC, Flynn TJ, Bradlaw JA, Roth WL, Barton CN, Yates JG. (2001). Pro-oxidant effects of the flavonoid myricetin on rat hepatocytes in culture. Toxicology Methods, 11 (4), 277-283. DOI: 10.1080/15376510109162663.

Salman KH, Mehany T, Zaki KG, Doury AMK (2024). Development of functional probiotic yogurt from buffalo milk supplemented with red beetroot (Beta vulgaris L.) as an antioxidant, natural colorant, and starter growth stimulant. Food Chemistry Advances, 5, 100776.

Schecter A, Colacino JA, Shah N, Harris TR, Papke O. (2009). Limitations in the use of potassium dichromate as a blood preservative for the analysis of organ halogenated compounds: two-month results. Chemosphere, 74 (3): 448-452. DOI: 10.1016/j.chemosphere.2008.09.044.

Scheidegger D, Pecora RP, Radici PM, Kivatinitz SC. (2010). Protein oxidative changes in whole and skim milk after ultraviolet or fluorescent light exposure. Journal of Dairy Science, 93 (11), 5101-5109. DOI: 10.3168/jds.2010-3513.

Sekhavatizadeh SS, Abadariyan N, Ebrahimi L, Hasanzadeh M. (2023). Effects of free and encapsulated Siah-e-Samarghandi grape seed extract on the physicochemical, textural, microbial, and sensorial properties of UF-Feta cheese. Food Science Nutrition, 11 (7): 3923-3938. DOI: 10.1002/fsn3.3378.

Selvaraj S, Amaral JM, Murty VR. (2022). Kinetics and antimicrobial activity of gallic acid by novel bacterial co-culture system using Taguchi's method and submerged fermentation. Archives of Microbiology, 204, 584. DOI: 10.1007/s00203-022-03168-2.

Sfakianakis P, Tzia C. (2014). Conventional and Innovative Processing of Milk for Yogurt Manufacture; Development of Texture and Flavor: A Review. Foods. 11;3(1):176-193.

Shakeel F, Haq N, Siddiqui NA. (2015) Solubility and thermodynamic function of vanillin in ten different environmentally benign solvents. Food Chemistry, 180: 244-248. DOI: 10.1016/j.foodchem.2015.01.102.

Sharma AD, Shivaprasad D, Chauhan K, Taneja NK. (2019). Control of E. coli growth and survival in Indian soft cheese (paneer) using multiple hurdles: Phytochemicals, temperature and vacuum. Food Science and Technology, 108350. DOI: 10.1016/j.lwt.2019.108350.

Sinaga H, Bansal N, Bhandari B. (2017). Effects of milk pH alteration on casein micelle size and gelation properties of milk. International Journal of Food Properties, 20 (1), 179-197. DOI: 10.1080/10942912.2016.1152480.

Smith NW, Fletcher AJ, Hill JP, McNabb WC. (2022). Modelling the contribution of milk to global nutrition. Frontiers Nutrition, 8: 716100. DOI: 10.3389/fnut.2021.716100.

Soares N, de Sá Silva C, Santiago-Silva P, Espitia P, Gonçalves M, Lopez M, Botrel D. (2009). Active and intelligent packaging for milk and milk products. Contemporary Food Engineering, 1, 175-199. DOI: 10.1201/9781420090390-c8.

Sobczak A, Danowska-Oziewicz M, Ząbek K, Miciński J, Narwojsz A. (2022). Effect of vitamin C fortification on the quality of cow's and goat's yoghurt. Food Science Nutrition, 18, 10 (11), 3621-3626. DOI: 10.1002/fsn3.2959.

Sorrentino E, Succi M, Tipaldi L, Pannella G, Maiuro L, Sturchio M, Coppola R, Tremonte P. (2018). Antimicrobial activity of gallic acid against food-related Pseudomonas strains and its use as biocontrol tool to improve the shelf life of fresh black truffles. International Journal of Food Microbiology, 266: 183-189. DOI: 10.1016/j.ijfoodmicro.2017.11.026.

The Indian Express (2024). Available on https://www.newindianexpress.com/states/tamil-nadu/2024/Aug/28/two-aavin-officials-in-madurai-suspended-for-milk-spoilage, accessed on 03-Feb-2025.

Tian Q, Wei S, Su H, Zheng S, Xu S, Liu M, Bo R, Li J. (2022a). Bactericidal activity of gallic acid against multi-drug resistance Escherichia coli. Microbial Pathogenesis, 173, 105824, DOI: 10.1016/j.micpath.2022.105824.

Tian L, Fu J, Wu M, Liao S, Jia X, Wang J, Yang S, Liu Z, Liu Z, Xue Z, Wang Y, Li H, Gong G. (2022b). Evaluation of gallic acid on membrane damage of Yersinia enterocolitica and its application as a food preservative in pork. International Journal of Food Microbiology, 2, 374, 109720. DOI: 10.1016/j.ijfoodmicro.

Tyl CE, Johansen SM, Kristoffersen AK, Devold GT, Brønner WK, Johansen A. (2024). Effect of storage temperature on the physicochemical and sensory properties of regular and lactose-reduced low-fat UHT milk. International Dairy Journal. 159: 106065, DOI: 10.1016/j.idairyj.2024.106065

Upadhyay N, Goyal A, Kumar A, Lal Ghai D, Singh R. (2014). Preservation of milk and milk products for analytical purposes. Food Reviews International, 30, 3, 203-224, DOI: 10.1080/87559129.2014.913292.

Vahedi M, Nasrolahei M, Sharif M, Mirabi AM. (2013). Bacteriological study of raw and unexpired pasteurized cow's milk collected at the dairy farms and super markets in Sari city in 2011. Journal of Preventive Medicine and Hygiene, 54 (2): 120-123.

Vaitkus V, Kazlauskaite E. (1977). Use of the Milko-Tester Mark-2 instrument for determination of milk fat. Trudy, Litovskii Filial Vsesoyuznogo Nauchno issledovatel skogo Instituta Maslodel noi i Syrodel noi Promyshlennosti, 11: 154, 127-133.

Valgas C, De Souza SM, Smânia EFA. (2007). Screening methods to determine antibacterial activity of natural products. Brazilian Journal of Microbiology, 38: 369-380.

Wüst J, Pischetsrieder M. (2016). Methionine sulfoxide profiling of milk proteins to assess the influence of lipids on protein oxidation in milk. Food & Function, 7 (6), 2526-2536. DOI: 10.1039/c5fo01550b.

Zajác P, Zubrická S, Čapla J, Zeleňáková L, Židek R, Čurlej J. (2016). Effect of preservatives on milk composition determination. International Dairy Journal, 61, 239-244. DOI: 10.1016/j.idairyj.2016.06.008.

Zamuz S, Munekata P, Dzuvor C, Zhang W, Sant'Ana A, Lorenzo J. (2021). The role of phenolic compounds against Listeria monocytogenes in food. A review. Trends in Food Science & Technology, 110, 385-392. DOI: 10.1016/j.tifs.2021.01.068..

Zheng M, Zhang C, Zhou Y, Lu Z, Zhao H, Bie X, Lu F. (2018). Preparation of gallic acid-grafted chitosan using recombinant bacterial laccase and its application in chilled meat preservation. Front Microbiology, 3 (9): 1729, DOI: 10.3389/fmicb.2018.01729.

Zheng Z, Jin Y, Zhang L, Xu X, Yang N. (2024). Inactivation of microorganisms in foods by electric field processing: A review. Journal of Agriculture and Food Research, 16, 101109.

Zou J, Liu X, Wang X, Yang H, Cheng J, Lin Y, Tang D. (2022). Influence of gelatin-chitosan-glycerol edible coating incorporated with chlorogenic acid, gallic acid and resveratrol on the preservation of fresh beef. Foods, 11 (23): 3813. DOI: 10.3390/foods11233813.

Kalaiselvan DHANUJA[1] – **Vellingiri VADIVEL**[1] ✉

DOI: https://doi.org./10.52091/EVIK-2025/2-2

Érkezett: 2025 május – Elfogadva: 2025 június

Fitokemikáliák hatása az élelmiszer-eredetű patogén baktériumokra, az oxidatív romlásra és a pasztőrözött tehéntej eltarthatóságára

Kulcsszavak: tejromlás; fitokemikáliák; antimikrobiális; antioxidáns; élelmiszer-eredetű kórokozók

1. Absztrakt

A tej nagyon érzékeny a mikrobiális szennyeződés és az oxidáció okozta romlásra. Ezek a romlási folyamatok rávilágítanak arra, hogy hatékony tartósítási módszerekre van szükség a tej eltarthatóságának meghosszabbításához, miközben megőrzik annak tápértékét. Ebben az összefüggésben az antibakteriális és antioxidáns tulajdonságokkal rendelkező fitokemikáliák hasznos szerepet játszhatnak a tej mikrobiális és oxidatív romlásának megelőzésében. A projekt célja az volt, hogy olyan fitokemikáliákat, mint a katechin, a kvercetin, a galluszsav, a szinapinsav, a betanin és a glicirretinsav antibakteriális hatását vizsgáljuk *E. coli* és *L. monocytogenes* baktériumokkal szemben.

Ezenkívül célunk volt meghatározni a kiválasztott vegyületek hatékonyságát a pasztőrözött tej eltarthatóságának meghosszabbításában. Az eredmények azt mutatták, hogy a galluszsav jelentős antibakteriális hatást fejtett ki, 18,66–13,00 mm közötti gátlási zónával (ZOI) és 18,37–36,74 mM közötti minimális gátló koncentrációval (MIC). Ennek következtében a galluszsavat választottuk ki további vizsgálatra antibakteriális mechanizmusainak feltárása céljából, pásztázó elektronmikroszkópos (SEM) elemzéssel. A galluszsav antioxidáns hatását DPPH-teszttel értékeltük, és vizsgáltuk a tejben található lipidek és fehérjék oxidációját gátló képességét.

Vizsgáltuk továbbá az érzékszervi profilt, a teljes baktériumszámot, valamint a malondialdehid (MDA) és a fehérje-karbonilok szintjét a galluszsavval kezelt, 4 napig 4 °C-on tárolt pasztőrözött tejben. Megfigyeltük, hogy a galluszsav károsítja az *E. coli* és a *L. monocytogenes* élelmiszer-kórokozók membránszerkezetét, amit pásztázó elektronmikroszkópos (SEM) felvételekkel igazoltunk. A DPPH gyökfogó aktivitás IC-50 értéke 43 µg/ml volt, a galluszsav 48,21%-kal gátolta az indukált lipidperoxidációt és 6,78%-kal a fehérjeoxidációt.

A kezelés negyedik napján a galluszsav jelentősen csökkentette a tejben található kolóniaalkotó egységek (CFU) számát 5,71 $\log_{10}$ CFU/ml-re, szemben a kálium-dikromát csoportban mért 6,17 $\log_{10}$ CFU/ml-rel. Ezenkívül a fehérje-karbonilok (1,33-szeres) és az MDA (2,06-szeres) gátlási szintje szignifikánsan magasabb volt a galluszsavval kezelt tejben, mint a kálium-dikromáttal kezelt mintákban. Ezeket a tényezőket figyelembe véve a galluszsav hatékony, biztonságos és környezetbarát tartósítószer lehet a tejipar számára.

[1] Kémiai biológia Labor (ASK-II-409), Kémiai és Biotechnológiai Kar, SASTRA Deemed Egyetem, Thanjavur, Tamilnadu, India

✉ Levelező szerző

Kalaiselvan DHANUJA		https://orcid.org/0009-0009-3850-104X
Vellingiri VADIVEL	vadivel@carism.sastra.edu	https://orcid.org/0000-0002-2709-433X

Aulia Putri Srie WARDANI[1], RAISYA[1], Aruzhan OTEGENOVA[2], Waminee NIRAMIT[2], Balázs P. SZABÓ[2], Judit KRISCH[2]

DOI: https://doi.org./10.52091/EVIK-2025/2-3

Arrived: June 2025 – Accepted: June 2025

Combined Effect of Natural Preservatives in Fruit Juices

Keywords: essential oil, lemon juice, fruit juices, natural preservatives

Abstract

The desire for natural preservatives is motivated by the demand for safer, healthier and more sustainable food products that meet modern consumer expectations. Lemon as natural preservatives in fruit juices have the potential to improve food safety, extend shelf life, and satisfy consumer demand for natural and healthy products due to its citric acid component and could potentially increase the antioxidant activity. Grapes and pear are nutritious fruits that can be found globally and are usually turned into juices for the everyday diet. The aim of this study is to examine the combined effect of two natural preservatives (lemon oil and lemon juice) in inhibiting microbial growth and extending the shelf life of juices compared to pasteurization. The juices and number measurements were taken at intervals of 0 days, 1 day, 2 days, 3 days, 1 week, and 4 weeks under chilled conditions. Other properties of the juices, such as pH, antioxidant activity, and sensory evaluation, were assessed once. The results suggest that lemon essential oil and lemon juice synergistically enhance the effect of pasteurization in fruit juices to extend their shelf life while maintaining their sensory qualities. Finally, lemon essential oil and lemon juice generally do not impair the acceptable organoleptic characteristics of fruit juices, except for the taste in grape juice.

[1] Department of Nutrition, Faculty of Sports and Health Sciences, Universitas Negeri Surabaya, Surabaya, Indonesia

[2] Department of Food Engineering, Faculty of Engineering, University of Szeged, Szeged, Hungary

Aulia Putri Srie WARDANI	auliawardani@unesa.ac.id	https://orcid.org/0009-0009-2829-4075
RAISYA		https://orcid.org/0009-0009-1988-7194
Aruzhan OTEGENOVA		https://orcid.org/0009-0009-3629-2643
Waminee NIRAMIT		https://orcid.org/0009-0009-7797-6849
Balázs P. SZABÓ		https://orcid.org/0000-0001-5950-3858
Judit KRISCH		https://orcid.org/0000-0002-6164-7794

Introduction

Fruit juices are highly susceptible to spoilage due to the proliferation of acid tolerant flora and their nutrient-rich composition. Some juices that have a pH level close to neutral, which provide ideal conditions for microbial growth, also have high risk. In addition to microbes, the presence of enzymes, oxygen, and other factors can also lead to undesirable changes in the sensory and nutritional quality of juices during storage. To ensure the microbiological safety and shelf life of fruit juices, various synthetic preservatives are commonly used in the food industry. Synthetic preservatives, such as sodium benzoate, potassium sorbate, and calcium propionate, which are widely used in the food industry to prevent microbial spoilage and extend the shelf life of food products, have been linked to adverse health effects, including triggering allergic reactions, weakening the immune system, causing cancer, hyperactivity, and other health problems (Sambu et al., 2022). As a result, consumers have become increasingly interested in natural and healthier alternatives to synthetic preservatives (Mesías et al., 2021). Lemon essential oil and lemon juice as natural preservatives have shown promise as potential alternatives to synthetic preservatives in food products. Citrus-based essential oils (EOs) are primarily extracted from the peels of citrus fruits, which are generally considered waste, making citrus-based EOs both economically valuable and environmentally beneficial (Agarwal et al., 2022). Additionally, the use of natural preservatives aligns with Sustainable Development Goal (SDG) 3, Good Health and Well-Being, and SDG 12, Responsible Consumption and Production. A growing consumer trend toward natural and eco-friendly products has also been reported (Mesías et al., 2021)

Limonene, a cyclic monoterpene and has the chemical formula ($C_{10}H_{16}$), is the main chemical component of citrus EOs, and it can range from 32% to 98% in amount (Neelima et al., 2017). Furthermore, a study by Tserennadmid et al. (2010) found that essential oils as anti-yeast had the maximum growth inhibition effect at the highest concentrations (2 µl/mL), with *Schizosaccharomyces pombe* as the most sensitive (0.0625-0.125µl/ml) and *Geotrichum candidum* as the most insensitive (0.5–2 µl/mL). A study by Yazgan et al. (2019) found that 10% lemon essential oil was effective against pathogens (mainly *Salmonella Paratyphi A*) and spoilage bacteria at a concentration of 25 mg/mL. Additionally, the acidity of lemon juice can help prevent enzymatic browning, which occurs when fruit juices are exposed to oxygen and can lead to color and flavor changes. A study of Bag et al. (2022) reported that 8% lemon juice could reduce enzyme activity by 39.73%. Some studies show that the minimum inhibitory concentration (MIC) values of lemon juice concentrate is 25 µg/mL for *Staphylococcus aureus*, 50 µg/mL for *Escherichia coli*, and 12.5 µg/mL for *Pseudomonas aeruginosa*. (Oikeh et al., 2016; Mishra et al., 2020). A combination of these natural preservatives may enhance their overall efficacy, making them a potential alternative to synthetic preservatives in fruit juices. In addition to their antimicrobial properties, these natural preservatives also possess antioxidant and flavoring properties, making them a multifunctional option for preserving the quality of food products (Bag et al., 2022; Olarinan 2020; Yazgan et al., 2019).

Generally, the need for natural preservatives is driven by the desire for safer, healthier, and more sustainable food products that meet the expectations of modern consumers. The development and use of natural preservatives in food products, including fruit juices, can potentially improve food safety and extend shelf life while also satisfying consumer demand for natural and healthy products. The aim of this study is to investigate the combined effect of natural preservatives (lemon essential oil and lemon juice) in grape and pear juices.

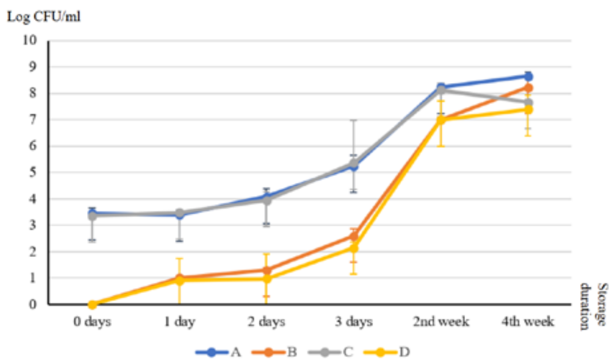


Figure 1. Total bacteria count in pear juice measured in log CFU/ml (left)

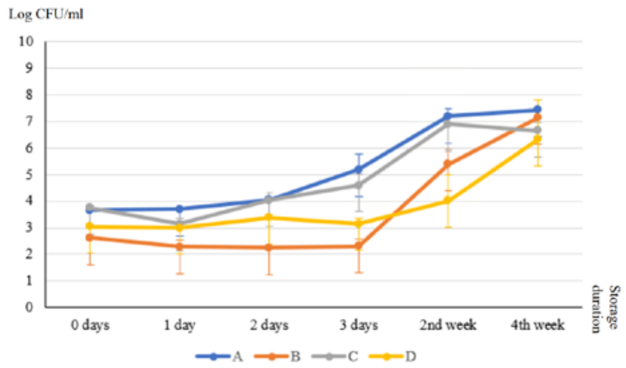


Figure 2. Total bacteria count in grape juice measured in log CFU/ml (right)

Materials and Methods

This experiment's main materials include pear, grapes, lemon essential oil and lemon juice. The lemon juice, as a natural preservative, was acquired by two bio-lemons that were squeezed, then filtered and later pasteurized at 60 °C for 30 min. This juice is stored in the refrigerator to maintain its quality. The lemon essential oil is commercially available lemon oil from Lecker's Bio Zitronenöl brand; it was extracted from lemon's peel.

The fruit juices were made from 1 kg of pear and 1 kg of white grapes acquired from the local market. Both pears and grapes were washed before turning them into juices using a cold press juicer (Braun J700 Multiquick7; Germany). The fresh juice was immediately treated. Grape juice was used without dilution and pear juice was diluted to 50% with sterilized water. Juices had no sugar supplementation. Both pear and grape juices were divided into four categories: 1) A is the untreated fresh juice; 2) B is heat treated at 80 °C for 30 minutes in a water bath; 3) C is with additional natural preservatives (0.25 µl/ml lemon essential oil and 200 µl pasteurized lemon juice) without any heat treatment; 4) D is the juices with additional natural preservatives (5 µl lemon essential oil and 200 µl lemon juice) combined with lower temperature heat treatment (60 °C for 30 min in a water bath). The concentration of additional essential oil and juice were taken from previous studies with consideration of the effectiveness of bacterial inhibition and sensory evaluation.

In order to evaluate the effectiveness of the natural preservatives, cell number measurements using malt extract medium (MEA) and tryptone glucose extract (TGE) were performed. These measurements were taken at intervals of 0 days, 1 day, 2 days, 3 days, 1 week, and 4 weeks under chilled storage conditions. Next, the pH was measured by a standardized pH meter. This pH meter was calibrated by standardized calibration solutions right before the measurement took place. The free radical scavenging activity of the samples was determined using 2,2-Diphenyl-1-picrylhydrazyl (DPPH obtained from Sigma-Aldrich, Germany) solution in 100 µM concentration prepared freshly before the measurement. The results were plotted as percent (%) DPPH radical scavenging capacity against the control:

Radical scavenging activity (%) = (A control - A sample)/A control) x 100

A sensory evaluation was also conducted by six untrained panelists with backgrounds in food science, including lab assistants, teachers, and students.

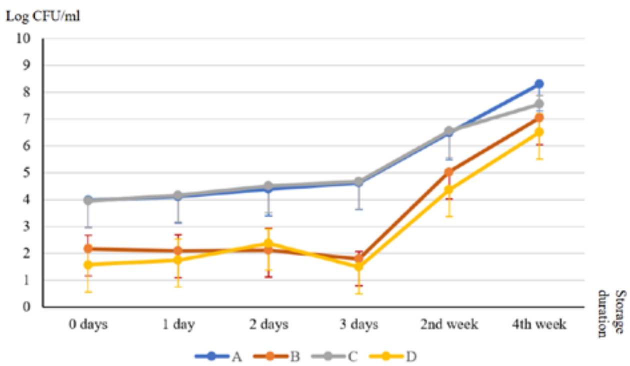


Figure 3. Total yeast count in pear juice measured in log CFU/ml (left)

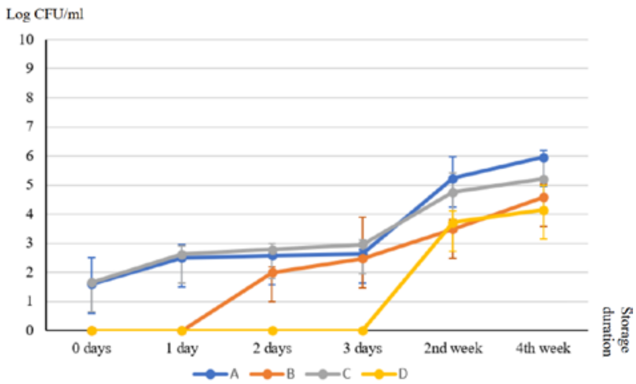


Figure 4. Total yeast count in grape juice measured in log CFU/ml (right)

Results and Discussion

pH measurements

As it can be seen in the Table 1, in pear juice, the pH ranges from 3.95 - 4.37. Notably, the categories that were added by lemon essential oil and lemon juice (C and D) tended to have lower pH rather than those without natural preservatives, with C (without heat treatment) being the lowest at 3.95. However, grape juice, which is naturally more acidic than pear, showed different results. All categories showed similarities in pH, interestingly only the C category had a slightly higher pH with 3.5 rather than 3.4.

Table 1. pH measurements for different treated pear and grape juices

pH of Juices	A (control)	B (pasteurized 80°C)	C (EO and juice)	D (heat treatment 60°C + EO and juice)
Pear juice	4.31 ± 1.02	4.37 ± 0.96	3.95 ± 0.6	4.02 ± 0.56
Grape juice	3.49 ± 0.36	3.42 ± 0.28	3.53 ± 0.3	3.45 ± 0.26

These results align with a study by Raj et al., (2011) that found that Asian pear juice, commonly referred to as sand pear, had a strong acidity and a pH level of about 4. Furthermore, grape juice also had a low pH level (often around 4). The level of acidity of fruits can vary depending on their organic acids. Grapes are typically more acidic than pear, because its organic acid content is predominantly by tartaric and malic acid content (Mato et al., 2005), which are stronger acids than citric acid which is found in pear and citrus fruit like lemon. Grapes also contain citric and succinic acids, albeit in very small amounts. All four species of pears in Sha et al. (2011) are mostly composed of malic and citric acids, with malic acid often being present in higher concentrations than citric acid. These acids contribute to the overall taste and acidity of the fruits. Furthermore, the pH and nutritional value of fruits and juices can also be influenced by its varieties, their ripeness (maturity), water content, and sugar content. It could also be influenced by environmental and technical factors (Raj et al., 2011).

The acidity of fruits can impact the microbial growth, as most spoilage and pathogenic bacteria and yeast grow in a neutral pH range 6.5-7.5. Therefore, if the pH of fruits falls outside this range, then the microbial growth could be inhibited. However, fruit juices with high acidity are susceptible to molds, yeast, lactic acid bacteria, thermo-acidophilic bacteria, and bacteria that can withstand acidity. (Mosqueda-Melgar et al., 2012; Unluturk and Atilgan, 2015)

Cell number measurements

The results of the total bacteria count of the pear and grape juices are presented in Figures 1 and 2. These figures illustrate the differences observed among the experimental groups.

Looking at Figures 1 and 2, the untreated pear and grape juices (A) showed the highest initial total bacteria count at 3.4 and 3.6 log CFU/ml, respectively. Fresh juice treated with natural preservatives (C) displayed a similar trend to A for both juices, but had fewer bacteria in the fourth week. The graphs demonstrate that pasteurization (B and D) resulted in a significant reduction percentage initially, especially for pear juice, where the first four measurements were below 3 log CFU/ml and this result was particularly notable in the category D (with the addition of lemon essential oil and lemon juice).

Furthermore, all treated fruit juices of both types maintained the limit for acceptable total bacteria count (5 log CFU/ml) set by the European Commission in 2005, for the first three days and up to two weeks for grape juice that underwent a combination of heat treatment and natural preservatives (D), which had a total bacteria count of 3.81 log CFU/ml. According to Bag et al. (2022), the upper limit of consumer acceptability in the case of juice for AMC (Aerobic mesophilic bacteria) is 6 log CFU/ml. However, the average total reduction for combination of heat treatment and natural preservatives was only 1.3 log CFU/ml, while the US FDA (2000) criteria for fruit and vegetable juice processing call for a 5-log reduction in the number of target pathogens of concern. (Unluturk and Atilgan, 2015).

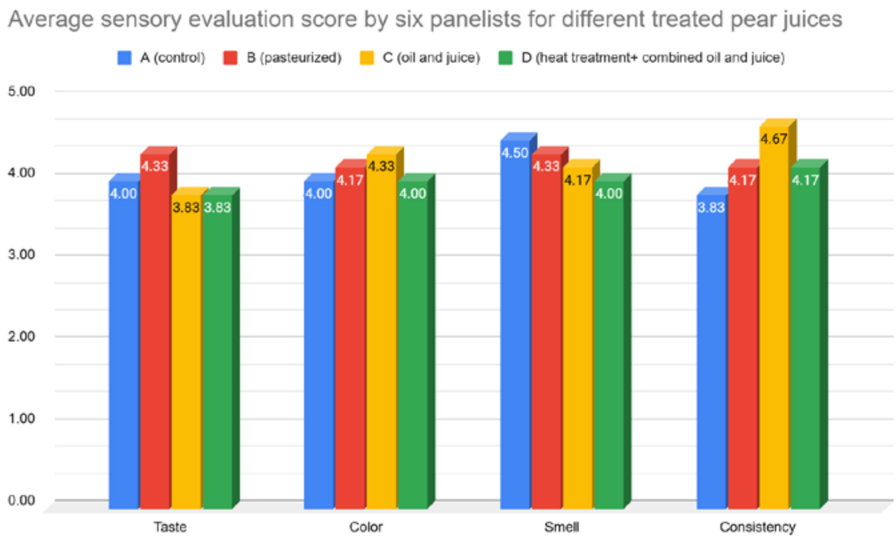


Figure 5. Average sensory evaluation score by six panelists for different treated pear juices

Furthermore, Figures 3 and 4 present the yeast counts of pear and grape juices measured over the course of the experiment.

Figures 3 and 4, depicting the yeast count, follow a similar pattern to Figures 1 and 2. Pasteurization (B and D) reduced the total yeast count to less than 3 log CFU/ml in the first three days for both juices. Notably, grape juice treated with a combination of natural preservatives and heat did not exhibit detectable yeasts in the first

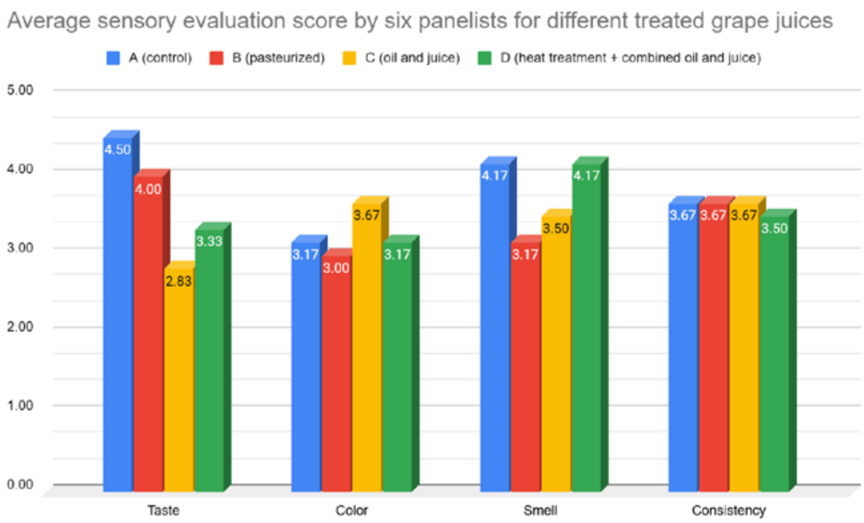


Figure 6. Average sensory evaluation score by six panelists for different treated grape juices

3 days and remained the least yeasty in the fourth week, accounting for 4.30 log CFU/ml. On the other hand, pasteurization only helped to diminish yeast growth in the first measurement in grape juice.

The results could be explained by the mechanism of action of natural preservatives combined and their combinations. Lemon essential oil eliminates Gram-positive bacteria better than Gram-negative ones, due to the difference in the bacterial cell wall's structure. Gram negative bacteria have a peptidoglycan monolayer surrounded by an outer membrane, which prevents the passage of small hydrophobic substances like the ingredients of lemon essential oils (Mishra et al, 2020). Limonene, a monoterpene, plays an important role as an antimicrobial in lemon essential oil (Han et al, 2021). Lemon juice has citric acid, flavonoids, limonoids, and saponin which could boost the effect of microorganism elimination in heat treatment by reducing internal pH, denaturing bacterial cell proteins, and damaging the cell membranes (Okeke et al., 2015; Ekawati and Darmanto, 2019). Even though lemon essential oils and lemon juice have various beneficial chemical compounds, studies have shown several higher concentrations in order to have a significant effect, for example a high concentration of citrus lemon juice (900 mg/mL) has been observed to significantly prevent bacteria that cause diarrhea in an Ekawati and Darmanto study (2019).

Another report implied lemon juice at 25 mg/mL can inhibit *Salmonella aureus* (Okeke et al., 2015). One study reported 200 µg/mL lemon juice concentrate could inhibit *Salmonella aureus*, however, this experiment uses the fresh juice concentrated and not in a fresh juice form. Lemon essential oil, on the other hand, has been reported to have an effect at a much lower concentration than that of lemon juice. For example, a study by Yazgan et al., 2019 revealed that 10% lemon essential oil was effective against pathogens (mainly *Salmonella Paratyphi A*) and spoilage bacteria at a dosage of 25 mg/mL. Another study by Tserennadmid et al. (2010) suggested that the essential oils as anti-yeast agents had the maximum growth inhibition effect at the highest concentrations of 2 µl/mL. Additionally, heat treatment with a temperature of 60°C to 75°C for 30 minutes could eliminate some of the microorganisms in fruit juices (Rupasinghe and Yu, 2012), while lemon essential oils and lemon juice could inhibit microorganisms via various action mechanisms.

Measurement of radical scavenging activity

After 4 weeks kept under chilled temperature, both juices' radical scavenging activity were assessed by the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay method. The DPPH (Sigma-Aldrich, Germany) solution at a concentration of 100 µM was prepared freshly before the measurement. The examination was measured at a two-times dilution, and the results presented in Table 2 are the mean of calculation for the undiluted fruit juices. The results are plotted as percentage (%) DPPH radical scavenging capacity against the control, which consisted of 1200 µL of 100 µM DPPH and 200 µL of 50% ethanol. This measurement is conducted to identify whether these fruit juices still have a high antioxidant level or not after 4 weeks.

Table 2. Radical scavenging activity for different treated pear and grape juices after 4 weeks (%)

Radical scavenging activity (%)	A (untreated)	B (pasteurized 80°C)	C (EO and juice)	D (heat treatment 60°C + EO and juice)
Pear juice	51.32 ± 7.2	43.05 ± 9.1	57.60 ± 14.9	44.12 ± 6.3
Grape juice	36.76 ± 4.1	46.88 ± 8.3	60.66 ± 1.8	55.30 ± 4.21

Radical scavenging activity refers to the juice's ability to neutralize or scavenge free radicals. It could be one of the indicators of a product's antioxidant level. As it can be seen in Table 2, the category C typically showed the highest level of radical scavenging activity in pear and grape juices. These results show that in this category pear juice neutralized about 57.6% of the DPPH radicals, while grape juice neutralized about 60.7%. Several compounds containing high antioxidant levels in lemon essential oil and lemon juice, such as ascorbic acid and flavonoids could be the explanation of these results (Neelima et al., 2017; Hanif et al. 2019). Furthermore, alkaloids, saponins, steroids, cardiac glycosides, terpenoids, and reducing sugar in citrus fruits could also increase the antioxidant activity (Oikeh et al., 2016). However, D was generally lower than C, due to the heat treatment that could potentially damage ascorbic acid in lemons and thus reduce its antioxidant activity (Rupasinghe and Yu, 2012). Pasteurized pear juice and untreated grape juice scored the lowest within its categories.

Sensory evaluation

Sensory evaluation was conducted by six untrained panelists who rated the taste, color, smell, and consistency of the juice on a scale of 1 to 5, with 1 being the least liked and 5 being the most liked. The results for the pear and grape juices are presented in Figures 5 and 6, respectively.

According to Figure 5 on pear juices, B had the highest score in the taste category (4.33), followed by A, while C and D both had the same score. For the color, C had a score of 4.33, making it the most preferred one by the panelists. The fact that the control (A) received the highest score (4.50) in the smell category and the heated pear juice with lemon juice and lemon essential oil (D) received the lowest score (4.00) shows that the addition of lemon oil and lemon juice with strong fragrance were not favoured. Lastly, in the consistency category, C was rated the highest (4.67), while untreated pear juice A was rated the lowest (3.83).

Although for taste and smell categories pear juice without any additional lemon juice and oil was more preferred (B had the highest score in taste and A had highest score in smell), however pear juice without heat treatment but with additional lemon essential oil and lemon juice (C) had the best attributes with 2 highest scores in color and consistency.

Figure 6 shows that there are differences in the taste category for grape juice. Whereas the fresh-untreated grape juice (A) scored the best with 4.50, the grape juice with a combination of lemon essential oil and lemon juice (C and D) typically scored the lowest with 2.83 and 3.33, respectively. However, this result contradicted the color category, with C having the highest score of 3.67 and B (pasteurized grape juice) having the lowest preference value with of only 3.00. Interestingly, unlike the pear juices' result, A (fresh-untreated grape juice) and D (heat treated with the addition of essential oil and lemon juice) scored the same in the color category (4.17). This result aligns with a study from Unluturk & Atilgan (2015) suggesting that while thermal treatment is a good way to improve the shelf life of juice, it also causes several qualitative and nutritional aspects to deteriorate, such as color change and loss of vital aroma and vitamins. Furthermore, the attributes of lemon essential oil and lemon juice to prevent enzymatic changes could be the reason why the color of these groups were more preferred. (Oikeh et al., 2016). Finally, for the consistency attribute, A, B, C had exactly the same value, which was 3.67 and D scored slightly less than the others with only 3.50.

To conclude, the organoleptic test resulted in the untreated fresh grape (A) juice scoring the highest, with a score of 3.88 out of 5. Meanwhile, both pasteurized pear juice (B) and fresh pear juice treated with lemon essential oil and juice (C) had the same highest score, with 4.25 out of 5. Furthermore, there is a consistent result for the color category, where both grape and pear juices in treatment C (addition of lemon essential oil and lemon juice without heat treatment) score the best, with 4.33 and 3.67, respectively. Based on these results, lemon essential oil and lemon juice generally do not impair the acceptable organoleptic characteristics of the fruit juices, except for the taste of grape juice.

Conclusions

These findings suggest that a combination of low dose (0.25 µl/ml) lemon essential oil and 1% (200 µl) lemon juice has little to no effect on the pH level of the juices and in inhibiting microbial growth in pear and grape juices. However, a mixture of lemon essential oil and lemon juice with the same concentration combined with low temperature for a long time (60°C in 30 minutes) possesses the same inhibition of microbial growth as pasteurization at 80°C in 30 minutes. Therefore, a combination of natural preservatives; lemon essential oil and lemon juice synergistically enhance the effect of pasteurization in fruit juices to extend their shelf life. However, radical scavenging activity is typically higher in pear juice and grape juice with natural preservatives only without heat treatment. For sensory evaluation, the color of the juices that have (0.25 µl/ml) lemon essential oil and 1% (200 µl) lemon juice scored the highest in both grape and pear juices. Lastly, these treatments do not have significant differences in the acceptable organoleptic characteristics of the fruit juices, except for the taste in grape juice. Finally, there is a need for further experiment using a higher concentration of lemon essential oil and lemon juice as natural preservatives to have a significant effect.

Acknowledgement

I (A.W) sincerely thank the Department of Food Engineering, Faculty of Engineering, University of Szeged, especially Dr. Judit Krisch, for her support and guidance. I also acknowledge the Stipendium Hungaricum Scholarship for funding this research.

References

Agarwal, P., Sebghatollahi, Z., Kamal, M., Dhyani, A., Shrivastava, A., Singh, K. K., Sinha, M., Mahato, N., Mishra, A. K., & Baek, K. H. (2022). Citrus essential oils in aromatherapy: Therapeutic effects and mechanisms. Antioxidants, 11(12), 2374.

Augusto, P.E., Tribst, A.A. and Cristianini, M., 2018. High hydrostatic pressure and high-pressure homogenization processing of fruit juices. In Fruit juices (pp. 393-421). Academic Press.

Bag, B.B., Panigrahi, C., Gupta, S. and Mishra, H.N., 2022. Efficacy of plant-based natural preservatives in extending shelf life of sugarcane juice: Formulation optimization by MOGA and correlation study by principal component analysis. Applied Food Research, 2(2), p.100164.

Barbosa, A.A.T., Mantovani, H.C. and Jain, S., 2017. Bacteriocins from lactic acid bacteria and their potential in the preservation of fruit products. Critical reviews in biotechnology, 37(7), pp.852-864.

Ekawati, E.R. and Darmanto, W., 2019. Lemon (*Citrus limon*) juice has antibacterial potential against diarrhea-causing pathogen. In IOP Conference Series: Earth and Environmental Science (Vol. 217, No. 1, p. 012023). IOP Publishing.

European Commission. (2005). Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. Official Journal of the European Union, L 338/1-26.

European Comission. 2022. Eurostat Statistics Explained. (https://ec.europa.eu/eurostat/statistics-explained/index.php?title=Vineyards_in_the_EU_-_statistics&oldid=566726)

European Comission. 2022. The pear market in the EU Production, yields and areas.

Han, Y., Chen, W. and Sun, Z., 2021. Antimicrobial activity and mechanism of limonene against Staphylococcus aureus. Journal of Food Safety, 41(5), p.e12918.

Hanif, M.A., Nisar, S., Khan, G.S., Mushtaq, Z. and Zubair, M., 2019. Essential oils. Essential Oil Research: Trends in Biosynthesis, Analytics, Industrial Applications and Biotechnological Production, pp.3-17.

Mato, I., Suárez-Luque, S. and Huidobro, J.F., 2005. A review of the analytical methods to determine organic acids in grape juices and wines. Food Research International, 38(10), pp.1175-1188.

Mesías, F.J., Martín, A. and Hernández, A., 2021. Consumers' growing appetite for natural foods: Perceptions towards the use of natural preservatives in fresh fruit. Food Research International, 150, p.110749.

Mishra, A.P., Devkota, H.P., Nigam, M., Adetunji, C.O., Srivastava, N., Saklani, S., Shukla, I., Azmi, L., Shariati, M.A., Coutinho, H.D.M. and Khaneghah, A.M., 2020. Combination of essential oils in dairy products: A review of their functions and potential benefits. Lwt, 133, p.110116.

Mosqueda-Melgar, J., Raybaudi-Massilia, R. M., & Martín-Belloso, O. (2012). Microbiological shelf life and sensory evaluation of fruit juices treated by high-intensity pulsed electric fields and antimicrobials. Food and Bioproducts Processing, 90(2), 205-214.

Nadeem, M., Ubaid, N., Qureshi, T. M., Munir, M., & Mehmood, A. (2018). Effect of ultrasound and chemical treatment on total phenol, flavonoids and antioxidant properties on carrot-grape juice blend during storage. Ultrasonics Sonochemistry, 45, 1–6. doi:10.1016/j.ultsonch.2018.02

Neelima Mahato, Kavita Sharma, Rakoti Koteswararao, Mukty Sinha, EkRaj Baral & Moo Hwan Cho (2017): Citrus essential oils: Extraction, authentication and application in food preservation, Critical Reviews in Food Science and Nutrition, DOI: 10.1080/10408398.2017.1384716

Oikeh, E.I., Omoregie, E.S., Oviasogie, F.E. and Oriakhi, K., 2016. Phytochemical, antimicrobial, and antioxidant activities of different citrus juice concentrates. Food science & nutrition, 4(1), pp.103-109.

Okeke, M.I., Okoli, A.S., Eze, E.N., Ekwume, G.C., Okosa, E.U. and Iroegbu, C.U., 2015. Antibacterial activity of *Citrus limonum* fruit juice extract. Pak J Pharm Sci, 28(5), pp.1567-1571.

Raj, D., Sharma, P.C. and Vaidya, D., 2011. Effect of blending and storage on quality characteristics of blended sand pear-apple juice beverage. Journal of food science and technology, 48, pp.102-105.

Rupasinghe, H.V. and Yu, L.J., 2012. Emerging preservation methods for fruit juices and beverages. Food additive, 22, pp.65-82.

Sambu S, Hemaram U, Murugan R, Alsofi A.A. 2022 Toxicological and Teratogenic Effect of Various Food Additives: An Updated Review. Biomed Res Int. 2022:6829409. doi: 10.1155/2022/6829409. PMID: 35782077; PMCID: PMC9249520.

Sha, S., Li, J., Wu, J. and Zhang, S., 2011. Characteristics of organic acids in the fruit of different pear species. African Journal of Agricultural Research, 6(10), pp.2403-2410.

Tserennadmid, R., Takó, M., Galgóczy, L., Papp, T., Pesti, M., Vágvölgyi, C., Almássy, K. and Krisch, J., 2011. Anti yeast activities of some essential oils in growth medium, fruit juices and milk. International Journal of Food Microbiology, 144(3), pp.480-486.

Unluturk, S. and Atilgan, M.R., 2015. Microbial safety and shelf life of UV-C treated freshly squeezed white grape juice. Journal of Food Science, 80(8), pp.M1831-M1841.

Yazgan, H., Ozogul, Y. and Kuley, E., 2019. Antimicrobial influence of nanoemulsified lemon essential oil and pure lemon essential oil on food-borne pathogens and fish spoilage bacteria. International Journal of Food Microbiology, 306, p.108266.

Aulia Putri Srie WARDANI[1], RAISYA[1], Aruzhan OTEGENOVA[2], Waminee NIRAMIT[2], Balázs P. SZABÓ[2], Judit KRISCH[2]

DOI: https://doi.org./10.52091/EVIK-2025/2-2

Érkezett: 2025 május – Elfogadva: 2025 június

A gyümölcslevekben található természetes tartósítószerek együttes hatása

Kulcsszavak: esszenciális olaj, citromlé, gyümölcslé, természetes tartósítószer

Absztrakt

A természetes tartósítószerek iránti igényt a biztonságosabb, egészségesebb és fenntarthatóbb élelmiszerek iránti kereslet motiválja, amelyek megfelelnek a modern fogyasztói elvárásoknak. A gyümölcslevekben természetes tartósítószerként használt citrom citromsavtartalmának köszönhetően javíthatja az élelmiszer-biztonságot, meghosszabbíthatja az eltarthatóságot, kielégítheti a fogyasztók természetes és egészséges termékek iránti igényét, és potenciálisan növelheti az antioxidáns hatást. A szőlő és a körte tápláló gyümölcsök, amelyek világszerte megtalálhatók, és általában gyümölcslevekké alakítják őket a mindennapi étrendben. A tanulmány célja annak vizsgálata, hogy két természetes tartósítószer (citromolaj és citromlé) együttes hatása milyen mértékben gátolja a mikrobiális növekedést és hosszabbítja meg a gyümölcslevek eltarthatóságát a pasztőrözéshez képest. A gyümölcsleveket 0, 1, 2, és 3 nap, 1 hét illetve 4 hét elteltével, hűtött körülmények között vizsgáltuk. A gyümölcslevek egyéb tulajdonságainak mérését, mint például a pH-érték, az antioxidáns hatás illetve az érzékszervi bírálat, csak egyszer végeztük el. Az eredmények arra utalnak, hogy a citrom illóolaj és a citromlé szinergikusan fokozza a pasztőrözés hatását a gyümölcslevekben, meghosszabbítva azok eltarthatóságát, miközben megőrzi érzékszervi tulajdonságaikat. Végül, a citrom illóolaj és a citromlé általában nem rontja a gyümölcslevek elfogadható érzékszervi tulajdonságait, kivéve a szőlőlé ízét.

[1] Táplálkozástudományi Tanszék, Sport- és Egészségtudományi Kar, Negeri Surabaya Egyetem, Surabaya, Indonézia

[2] Élelmiszermérnöki Intézet, Mérnöki Kar, Szegedi Tudományegyetem, Szeged, Magyarország

Aulia Putri Srie WARDANI	auliawardani@unesa.ac.id	https://orcid.org/0009-0009-2829-4075
RAISYA		https://orcid.org/0009-0009-1988-7194
Aruzhan OTEGENOVA		https://orcid.org/0009-0009-3629-2643
Waminee NIRAMIT		https://orcid.org/0009-0009-7797-6849
Balázs P. SZABÓ		https://orcid.org/0000-0001-5950-3858
Judit KRISCH		https://orcid.org/0000-0002-6164-7794

Mustafa SEViNDiK[1]

DOI: https://doi.org./10.52091/EVIK-2025/2-4

Arrived: June 2025 – Accepted: June 2025

Evaluation of Antioxidant, Anticholinesterase and Antiproliferative Effects of Ethanol Extract of Terfezia Olbiensis

Keywords: *Terfezia olbiensis,* antioxidant activity, anticholinesterase activity, antiproliferative activity, biological activities

Abstract

In this study, antioxidant, anticholinesterase and antiproliferative activities of ethanolic extract of *Terfezia olbiensis* (Tul. & C. Tul.) Sacc. mushroom were evaluated. Antioxidant activity was determined by total antioxidant capacity (TAS), total oxidant capacity (TOS) and oxidative stress index (OSI) parameters and TAS value was calculated as 1.983±0.049 mmol/L, TOS value as 16.762±0.453 μmol/L and OSI value as 0.846±0.043. In addition, acetylcholinesterase activity and butyrylcholinesterase activity of *T. olbiensis* were found as 91.94±1.75 μg/mL and 132.08±2.09 μg/mL. Finally, the antiproliferative effect of *T. olbiensis* on the A549 lung cancer cell line was investigated and it was determined that the extract decreased cell viability in a dose-dependent manner. Our study results indicate that *T. olbiensis* can be evaluated as a potential anticancer agent in lung cancer cells. These findings suggest that *T. olbiensis* may be an important source for future therapeutic applications with its biological activities that show positive effects on health. However, these activities need to be confirmed with more detailed molecular and *in vivo* studies.

[1] Department of Biology, Faculty of Engineering and Nature Sciences, Osmaniye Korkut Ata University

Mustafa SEViNDiK sevindik27@gmail.com https://orcid.org/0000-0001-7223-2220

Introduction

Medicinal mushrooms are natural resources that attract great attention in the field of health thanks to the bioactive components they contain. These mushrooms, which have been used in the treatment of various diseases for centuries in traditional medicine, are increasingly being studied in terms of their effectiveness and benefits with modern scientific research (Sevindik et al., 2023). They attract attention with their versatile effects, especially strengthening the immune system, reducing oxidative stress, supporting cell renewal and preventing some chronic diseases. Polysaccharides, triterpenes, sterols, phenolic compounds and various enzymes in their structures are the main substances that constitute the pharmacological properties of mushrooms (Saridogan et al., 2021). Thanks to these components, medicinal mushrooms have been reported in scientific studies to have anti-inflammatory, antioxidant, antimicrobial, antitumor and neuroprotective effects (Hetland et al., 2020; Cör Andrejč et al., 2022; Gürgen and Sevindik, 2022; Bal et al., 2023). In addition, they are used as a supplement in both traditional and modern treatment approaches due to their anti-stress, anti-fatigue and anti-aging effects (Krupodorova and Sevindik, 2020). With the increasing interest in natural products in recent years, medicinal mushrooms have begun to play an important role in the development of both food supplements and pharmaceutical formulations. This highlights them as functional biological agents with both preventive and therapeutic potential (Wasser, 2011; Islek et al., 2021; Venturella et al., 2021). In this study, antioxidant, anticholinesterase and antiproliferative activities of *Terfezia olbiensis* were determined.

Terfezia spp is an underground mushroom that draws attention with its high nutritional value and rich chemical composition. It contains approximately 16% protein, 28% carbohydrates, 4% crude fiber and 2% crude fat; in addition, nine saturated and four unsaturated fatty acids and 29 different amino acids were identified (Bokhary and Parvez, 1993). In addition, it is rich in macro elements such as sodium (Na) and potassium (K), as well as micro elements such as iron (Fe), copper (Cu) and nickel (Ni) (Hadjira et al., 2024). Various extracts of *T. claveryi* show significant antimicrobial activity against both gram-positive and gram-negative bacteria. It has been reported that especially the methanolic extract is effective against bacteria such as *Bacillus subtilis* and *Staphylococcus* aureus (Khojah et al., 2022). Its antioxidant potential has been evaluated by methods such as DPPH, FRAP and ABTS, and it has been shown to have a high level of radical scavenging capacity (Dahham et al., 2018; İnci and Kırbağ, 2018). This species, which is widely used in traditional medicine for the treatment of diabetes, has been determined to exhibit hypoglycemic effects in *in vitro* and *in vivo* models; It has been determined that it reduces blood glucose levels, especially in high-fat diet-induced diabetic mice (Veeraraghavan et al., 2022). In addition, it has been reported to exhibit moderate anticancer activity against various cancer cell lines (Dahham et al., 2018). These versatile biological activities indicate that *T. olbiensis* is a remarkable natural resource for future evaluation in clinical studies and use in therapeutic applications.

2. Materials and Methods

Terfezia olbiensis samples used in this study were obtained from Gaziantep province of Turkey. Collected mushroom samples were dried under laboratory conditions and prepared in a form suitable for analysis. For the extraction process, 10 grams of dried mushroom sample was extracted using a Soxhlet device. 250 mL of ethanol was preferred as the solvent during extraction and the process was carried out for approximately 6 hours at 50 °C. The obtained crude extract was concentrated with a Buchi R100 Rotary Evaporator operating at 40 °C to completely remove the solvent. Afterwards, the obtained extracts were stored at +4 °C until the analysis was completed.

Antioxidant activity test

The total antioxidant capacity (TAS) and total oxidant level (TOS) of the ethanol extract used in the study were determined by commercial analysis kits belonging to Rel Assay Diagnostics. The measurements were carried out in accordance with the protocol instructions provided by the manufacturer. In TAS analyses, trolox was used as a standard substance, while in TOS analyses, hydrogen peroxide was taken as a reference for calibration purposes. The obtained TAS and TOS results were expressed in mmol/L and µmol/L, respectively (Erel, 2004; Erel, 2005). Oxidative Stress Index (OSI) was calculated by converting both parameters to common units and dividing the TOS value by TAS (Sevindik, 2019).

Anticholinesterase activity test

In this study, the anticholinesterase effect of ethanolic extract was investigated based on the method developed by Ellman et al. (1961). Inhibitory effects on acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes were evaluated, and galantamine was used as a reference inhibitor in comparative analyses. Before experimental applications, stock solutions were prepared by diluting the extracts to different concentrations in the range of 200–3.125 µg/mL. During the analysis, 130 µL of 0.1 M phosphate buffer (pH 8.0), 10 µL of extract solution and 20 µL of target enzyme (AChE or BChE) were added to the 96-well microplates, respectively. The plates were incubated at 25 °C under dark conditions for 10 min. At the end of the incubation period, 20 µL of DTNB [5,5′-dithiobis-(2-nitrobenzoic acid)] solution and 20 µL of substrate (acetylcholine iodide or butyrylcholine iodide) were added to each well to initiate the reaction. Enzymatic activity was measured spectrophotometrically at 412 nm wavelength. The inhibitory effects of the extracts were determined by calculating the IC_{50} (µg/mL) values, and all analyses were carried out with three independent replicates.

Antiproliferative activity test

In this study, the cytotoxic effects of the ethanolic extract on the A549 lung cancer cell line were evaluated by the cell viability test based on MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide). Before the experimental applications, the extract was prepared at different concentrations as 25, 50, 100 and 200 µg/mL. When the cells reached approximately 70–80% confluence, they were treated with 3.0 mL of Trypsin-EDTA solution (Sigma-Aldrich, MO, USA) to detach them from the surface. The detached cells were transferred to culture plates and subjected to a 24-hour incubation period. At the end of this period, extracts at determined concentrations were applied to the cells and a second 24-hour incubation was performed. After the process, the supernatant medium was removed and MTT solution prepared at a concentration of 1 mg/mL was added to the cells and incubated at 37 °C. Dimethyl sulfoxide (DMSO) (Sigma-Aldrich, MO, USA) was added to each well to dissolve the purple formazan crystals formed by reduction by MTT. Cell viability was measured with an Epoch model spectrophotometer (BioTek Instruments, Winooski, VT, USA) operating at a wavelength of 570 nm and evaluated according to the absorbance values obtained (Bal et al., 2017).

Results and discussion

Antioxidant activity

Oxidative stress is a condition that causes damage to cells as a result of the imbalance of free radicals and reactive oxygen species (ROS) in the body. This process is one of the main factors of aging, cellular damage, inflammation and many chronic diseases (Abdelkader et al., 2024). Antioxidants provide an important protective mechanism as compounds that balance oxidative stress and neutralize free radicals (Arslan et al., 2025). Mushrooms have a strong protective effect with natural antioxidants such as phenolic compounds, flavonoids, tocopherols and ascorbic acid (Sevindik, 2021). These compounds help prevent damage caused by oxidants (free radicals and reactive oxygen species) by protecting cells from oxidative damage (Krupodorova et al., 2022). These antioxidant properties offer a potential protective effect in the prevention of diseases such as aging, cardiovascular diseases and cancer by reducing cellular damage caused by oxidative stress. In this study, antioxidant potential of *T. olbiensis* was evaluated. The obtained data are presented in Table 1.

Table 1. TAS, TOS ve OSI values of Terfezia olbiensis

Sample	TAS mmol/L	TOS µmol/L	OSI (TOS/(TASx10))
Terfezia olbiensis	1.983±0.049	16.762±0.453	0.846±0.043

Values are presented as mean±SD

In the literature, the TAS value of the methanol extract of *Terfezia claveryi* was reported as 1.18 and the TOS value as 3.45 (İnci and Kırbağ, 2018). In our study, the ethanol extract of *T. olbiensis* was studied. Since the ethanol extract was used in our study, it should be considered that this different solvent may have created different effects on the solubility and activity of antioxidant and oxidant compounds. Solvents such as ethanol can increase the antioxidant capacity by increasing the solubility of some active compounds (Tang et al., 2024). This may have contributed to the higher TAS and TOS values obtained in our study. In addition, TAS, TOS and OSI values of different mushroom species have been reported in the literature. A different *Terfezia species*, *Terfezia boudieri*, has a TAS value of 2.332 mmol/L, TOS value of 26.945 µmol/L and OSI value of 1.156 reported (Sevindik et al., 2018). Otidea onotica has a TAS value of 8.866 mmol/L, TOS value of 14.724 µmol/L and OSI value of 0.166 reported (Sevindik et al., 2025). Phellinus hartigii has a TAS value of 4.98 mmol/L, TOS value of 9.27 µmol/L and OSI value of 0.19 reported (Ünal et al., 2025). *Lactarius deliciosus* has a TAS value of 7.468 mmol/L, TOS value of 13.161 µmol/L and OSI value of 0.176 reported (Gürgen and Sevindik, 2025). The TAS value of *Hericium erinaceus* was reported as 5.426 mmol/L, TOS value as 6.621 µmol/L and OSI value as 0.122 (Sevindik et al., 2024). Compared to these studies, the TAS value of *T. olbiensis* used in our study was determined to be lower than *T. boudieri*, *O. onotica*, *P. hartigii*, *L. deliciosus* and *H. erinaceus*. The TAS value is an indicator of the whole of the antioxidant compounds produced in natural products (Sabik et al., 2024). The TOS value is an indicator of the whole of the oxidant compounds produced in natural products. The OSI value shows the percentage of suppression of oxidant compounds by antioxidant compounds (Sabik et al., 2024). The TOS and OSI values of *T. olbiensis* used in our study were determined to be lower than *T. boudieri* and higher than *O. onotica*, *P. hartigii*, *L. deliciosus* and *H.*

erinaceus. In conclusion, the obtained data show that the antioxidant and oxidant properties of *T. olbiensis* are diverse when compared to different mushroom species and that the biological potential of this species can be the subject of further research. The examination of TAS, TOS and OSI values provides comprehensive information about the effects of natural products on health and helps us understand how the use of different solvents can affect biological activities.

Anticholinesterase activity

Mushrooms stand out as a potential biological agent in the treatment of neurological diseases by exhibiting anticholinesterase activity. These activities are particularly associated with the inhibition of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes. While AChE reduces acetylcholine levels in the synaptic cleft by hydrolyzing neurotransmitters such as acetylcholine, BChE also plays a similar role in the metabolism of neurotransmitters (Liuzzi et al., 2023). Inhibiting AChE and BChE activities may be an important strategy in the treatment of neurological disorders such as Alzheimer's and Parkinson's diseases. The bioactive compounds contained in mushrooms increase the levels of neurotransmitters such as acetylcholine and butyrylcholine by inhibiting these enzymes and strengthen synaptic transmission (Obaid et al., 2022). This effect supports the anti-Alzheimer properties of mushrooms and is considered as a potential therapeutic approach for the treatment of neurological disorders (Abitbol et al., 2022). In this study, the anticholinesterase activity of *T. olbiensis* was investigated and the results obtained are presented in Table 2.

Table 2. Anti-AChE and anti-BChE values of *Terfezia olbiensis*

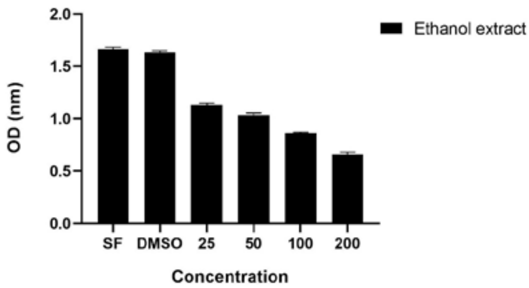
Sample	AChE µg/mL	BChE µg/mL
Terfezia olbiensis	91.94±1.75	132.08±2.09
Galantamine	7.72±0.39	16.67±0.20

* The values were studied in 3 replicates and the std values are given.

In this study, the anticholinesterase effect of *T. olbiensis* ethanol extract was evaluated and the obtained results, although lower than the inhibitory potential of the reference inhibitor galantamine, reveal that *T. olbiensis* has a significant cholinesterase inhibitory capacity. Inhibition of cholinesterase enzymes is considered an important therapeutic strategy in the treatment of neurodegenerative disorders related to the cholinergic system, especially Alzheimer's disease. In this context, the discovery of naturally derived cholinesterase inhibitors is of both scientific and clinical value (de Oliveira et al., 2024). In the literature, it is reported that hexane, ethyl acetate, methanol and water extracts of *T. claveryi* have IC_{50} values ranging from 100.45–287.66 µg/mL against AChE enzyme and 41.50–289.74 µg/mL against BChE enzyme (Kıvrak, 2015). The use of different solvents in these studies significantly affected the chemical composition of the extracts and therefore the enzyme inhibition profiles. The ethanol extract of *T. olbiensis* used in our study has a lower IC_{50} value of 91.94 µg/mL for AChE and shows higher inhibitory potential than some fractions in the study of Kıvrak (2015). This suggests that ethanol may be an effective solvent in the extraction of active components from *T. olbiensis* and that the obtained extract may create a significant inhibition on cholinesterase enzymes. The stronger inhibition observed against AChE suggests that the extract may be more selective against this enzyme. In this context, the interaction of phenolic compounds, flavonoids or terpenoids contained in the extract with both AChE and BChE enzymes should be examined at the molecular level. In addition, comparative analyses of extracts prepared with different solvents will be guiding in determining which fractions are more effective. In conclusion, the ethanol extract of *T. olbiensis* shows an effective inhibitory activity especially on AChE. These findings indicate that this species may be a source of natural compounds with anticholinesterase properties and has the potential to be evaluated in drug development studies for neurodegenerative diseases in the future.

Antiproliferative activity

Mushrooms are important natural resources that exhibit antiproliferative properties due to the bioactive compounds they contain. Their antiproliferative activities show effects aimed at inhibiting the growth and proliferation of cancer cells (Wasser, 2002). This feature is considered as a potential therapeutic approach, especially in cancer treatment. The antiproliferative effect of mushrooms is being investigated with in vitro experiments on various cell lines. In addition, compounds such as polysaccharides, phenolic compounds and terpenoids found in mushrooms have been shown to inhibit cell growth and prevent metastasis of cancer cells (Park et al., 2024). The antiproliferative activities of mushrooms hold great hope for the discovery of new natural therapeutic agents that can be used in cancer treatments in the future (Wasser, 2017). In this study, the effects of *T. olbiensis* on A549 lung cancer cells were examined and the results obtained are presented in Figure 1 as µg/mL.



** Control group: Cells were maintained only in standard medium and were not exposed to any chemical treatments, DMSO group: As a solvent control, only DMSO was added to the medium; no extract was applied, Treatment groups: Extract solutions prepared at concentrations of 25, 50, 100, and 200 µg/mL were applied to the cells.*

Figure 1. Antiproliferative activity of Terfezia olbiensis

In this study, the antiproliferative effect of ethanol extract of *T. olbiensis* on A549 lung adenocarcinoma cell line was evaluated. The results presented in Figure 1 show that increasing extract concentrations caused a significant and dose-dependent decrease in cell viability. While the high OD values observed in SF (serum free) and DMSO control groups revealed that the cells maintained healthy proliferation, the gradual decrease in OD values in the extract-applied groups clearly reflected the antiproliferative activity of the extract. This effect became more pronounced especially at concentrations of 100 and 200 µg/mL, indicating that the biological activity was dose-dependent. It has been previously reported in the literature that Terfezia claveryi exhibited cytotoxic effects on U-87 MG glioblastoma and MCF-7 breast cancer cell lines (Khadri et al., 2017; Dahham et al., 2018). However, there is no evidence in the literature regarding the effects of *T. olbiensis* against the A549 human lung cancer cell line. The findings obtained in this study are antiproliferative activity evaluation on this species and fill an important gap in the existing literature. However, advanced molecular and in vivo studies are needed to confirm these initial findings and to elucidate the mechanisms of action in more detail.

Conclusion

In this study, antioxidant, anticholinesterase and antiproliferative activities of ethanol extract of *T. olbiensis* mushroom were evaluated and the obtained findings revealed the biological potential of this species. Ethanol extract of *T. olbiensis* showed a moderate effect in terms of antioxidant capacity, especially its inhibitory effect on AChE enzyme was remarkable. In addition, *T. olbiensis* was found to have an antiproliferative effect on A549 lung cancer cell line, indicating that this species can be evaluated as a potential anticancer agent. These findings suggest that *T. olbiensis* may be a natural source that provides positive effects on health and may contribute to future treatment methods. In the future, more detailed examination of the chemical structures and action mechanisms of the active components contained in *T. olbiensis* will provide a better understanding of the biological activities of this species. In particular, investigation of its antioxidant and anticholinesterase properties as well as its anticancer effects at the molecular level may further strengthen the potential therapeutic use of this mushroom. In addition, comparative analyses of extractions with different solvents will be an important step in determining the most effective compounds and fractions. Confirmation of these findings with in vivo studies and clinical trials may reveal the future usability of *T. olbiensis* as a therapeutic option in areas such as neurological diseases and cancer treatment.

References

Abdelkader, M. A. E., Mediatrice, H., Lin, D., Lin, Z., & Aggag, S. A. (2024). Mitigating Oxidative Stress and Promoting Cellular Longevity with Mushroom Extracts. Foods, 13(24), 4028.

Abitbol, A., Mallard, B., Tiralongo, E., & Tiralongo, J. (2022). Mushroom natural products in neurodegenerative disease drug discovery. Cells, 11(23), 3938.

Arslan, N. P., Dawar, P., Albayrak, S., Doymus, M., Azad, F., Esim, N., & Taskin, M. (2025). Fungi-derived natural antioxidants. Critical Reviews in Food Science and Nutrition, 65(9), 1593–1616.

Bal, C., Akgul, H., Sevindik, M., Akata, I., & Yumrutas, O. (2017). Determination of the anti-oxidative activities of six mushrooms. Fresenius Envir Bull, 26(10), 6246–6252.

Bal, C., Eraslan, E. C., & Sevindik, M. (2023). Antioxidant, antimicrobial activities, total phenolic and element contents of wild edible mushroom Bovista nigrescens. Prospects in Pharmaceutical Sciences, 21(2), 37–41.

Bokhary, H. A., & Parvez, S. (1993). Chemical composition of desert truffles Terfezia claveryi. Journal of Food Composition and Analysis, 6(3), 285–293.

Cör Andrejč, D., Knez, Ž., & Knez Marevci, M. (2022). Antioxidant, antibacterial, antitumor, antifungal, antiviral, anti-inflammatory, and nevro-protective activity of Ganoderma lucidum: An overview. Frontiers in pharmacology, 13, 934982.

Dahham, S. S., Al-Rawi, S. S., Ibrahim, A. H., Majid, A. S. A., & Majid, A. M. S. A. (2018). Antioxidant, anticancer, apoptosis properties and chemical composition of black truffle Terfezia claveryi. Saudi journal of biological sciences, 25(8), 1524–1534.

de Oliveira, P. C. O., da Silva, G. M., Cass, Q. B., de Moraes, M. C., & Cardoso, C. L. (2024). Natural products as a source of cholinesterase inhibitors. Pharmacological Research-Natural Products, 5, 100099.

Ellman, G. L., Courtney, K. D., Andres Jr, V., & Featherstone, R. M. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. Biochemical pharmacology, 7(2), 88–95.

Erel O. 2004. A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. Clinical biochemistry, 37(4): 277–285.

Erel O. 2005. A new automated colorimetric method for measuring total oxidant status. Clinical biochemistry, 38(12): 1103–1111.

Gürgen, A., & Sevindik, M. (2022). Application of artificial neural network coupling multiobjective particle swarm optimization algorithm to optimize Pleurotus ostreatus extraction parameters. Journal of Food Processing and Preservation, 46(11), e16949.

Gürgen, A., & Sevindik, M. (2025). Single and Multi-Objective Optimization of the Red Pine Mushroom Lactarius deliciosus (Agaricomycetes) Extraction Conditions Using Artificial Intelligence Methods and Biological Activities of Optimized Extracts. International Journal of Medicinal Mushrooms, 27.

Hadjira, G., Bakchiche, B., Erenler, R., Yildiz, İ., Mohamed, A. S., & El-Shazly, M. A. (2024). Mineral contents, antioxidant and antimicrobial activities of algerian Terfezia Claveryi extracts. Fabad Journal of Pharmaceutical Sciences, 49(1), 19–36.

Hetland, G., Tangen, J. M., Mahmood, F., Mirlashari, M. R., Nissen-Meyer, L. S. H., Nentwich, I., Therkelsen, S. P., Tjønnfjord, G. E., & Johnson, E. (2020). Antitumor, anti-inflammatory and antiallergic effects of Agaricus blazei mushroom extract and the related medicinal basidiomycetes mushrooms, Hericium erinaceus and Grifola frondosa: A review of preclinical and clinical studies. Nutrients, 12(5), 1339.

Islek, C., Saridogan, B. G. O., Sevindik, M., & Akata, I. (2021). Biological activities and heavy metal contents of some Pholiota species. Fresenius Environmental Bulletin, 30(6), 6109–6114.

İnci, Ş., & Kırbağ, S. (2018). Terfezia claveryi Chatin'in besinsel içeriği, antioksidan ve antimikrobiyal aktivitesi. Artvin Çoruh Üniversitesi Orman Fakültesi Dergisi, 19(2), 138–143.

Khadri, H., Aldebasi, Y. H., & Riazunnisa, K. (2017). Truffle mediated (Terfezia claveryi) synthesis of silver nanoparticles and its potential cytotoxicity in human breast cancer cells (MCF-7). African Journal of Biotechnology, 16(22), 1278–1284.

Khojah, H. M., Abdelhalim, O. B., Mostafa, M. A., & Habib, E. S. E. (2022). Antimicrobial efficacy of extracts of Saudi Arabian desert Terfezia claveryi truffles. Saudi Journal of Biological Sciences, 29(11), 103462.

Kıvrak, İ. (2015). Analytical methods applied to assess chemical composition, nutritional value and in vitro bioactivities of Terfezia olbiensis and Terfezia claveryi from Turkey. Food Analytical Methods, 8, 1279–1293.

Krupodorova, T., & Sevindik, M. (2020). Antioxidant potential and some mineral contents of wild edible mushroom Ramaria stricta. AgroLife Scientific Journal, 9(1), 186–191.

Krupodorova, T., Barshteyn, V., & Sevindik, M. (2022). Antioxidant and antimicrobial potentials of mycelial extracts of Hohenbuehelia myxotricha grown in different liquid culture media. BioTechnologia, 103(1), 19–28.

Liuzzi, G. M., Petraglia, T., Latronico, T., Crescenzi, A., & Rossano, R. (2023). Antioxidant compounds from edible mushrooms as potential candidates for treating age-related neurodegenerative diseases. Nutrients, 15(8), 1913.

Obaid, R. J., Naeem, N., Mughal, E. U., Al-Rooqi, M. M., Sadiq, A., Jassas, R. S., Moussa, Z., & Ahmed, S. A. (2022). Inhibitory potential of nitrogen, oxygen and sulfur containing heterocyclic scaffolds against acetylcholinesterase and butyrylcholinesterase. RSC Advances, 12(31), 19764–19855.

Park, G. S., Shin, J., Hong, S., Gopal, J., & Oh, J. W. (2024). Anticarcinogenic potential of the mushroom polysaccharide lentinan on gastric and colon cancer cells: Antiproliferative, antitumorigenic, Mu-2-related death-inducing gene, MUDENG ramifications. Journal of Industrial and Engineering Chemistry, 135, 122–130.

Sabik, A. E., Sevindik, M., Mohammed, F. S., & Dogan, M. (2024). A New Natural Source against A549 Lung Cancer Cells Anthemis cotula and its Biological Activities and Phenolic Contents. Pharmaceutical Chemistry Journal, 1–7.

Saridogan, B. G. O., Islek, C., Baba, H., Akata, I., & Sevindik, M. (2021). Antioxidant antimicrobial oxidant and elements contents of Xylaria polymorpha and X. hypoxylon (Xylariaceae). Fresenius Environmental Bulletin, 30(5), 5400–5404.

Sevindik, M. (2019). The novel biological tests on various extracts of Cerioporus varius. Fresenius Environmental Bulletin, 28(5), 3713–3717.

Sevindik, M. (2021). Anticancer, antimicrobial, antioxidant and DNA protective potential of mushroom Leucopaxillus gentianeus (Quél.) Kotl. Indian Journal of Experimental Biology (IJEB), 59(05), 310–315.

Sevindik, M., Bal, C., Eraslan, E. C., Uysal, I., & Mohammed, F. S. (2023). Medicinal mushrooms: a comprehensive study on their antiviral potential. Prospects in Pharmaceutical Sciences, 21(2), 42–56.

Sevindik, M., Bal, C., Krupodorova, T., Gürgen, A., & Eraslan, E. C. (2025). Extract optimization and biological activities of Otidea onotica using Artificial Neural Network-Genetic Algorithm and response surface methodology techniques. BMC biotechnology, 25, 25.

Sevindik, M., Gürgen, A., Khassanov, V. T., & Bal, C. (2024). Biological activities of ethanol extracts of Hericium erinaceus obtained as a result of optimization analysis. Foods, 13(10), 1560.

Sevindik, M., Pehlivan, M., Dogan, M., & Selamoglu, Z. (2018). Phenolic content and antioxidant potential of Terfezia boudieri. Gazi University Journal of Science, 31(3), 707–711.

Tang, X., Zhang, Y., Wang, L., Sun, C., Wang, L., & Huang, Z. (2024). Excited-state antioxidant capacity of Flavonoids based on solvent effect: A TD-DFT study. Journal of Molecular Liquids, 404, 124973.

Ünal, O., Gürgen, A., Krupodorova, T., Sevindik, M., Kabaktepe, Ş., & Akata, I. (2025). Optimization of Phellinus hartigii extracts: Biological activities, and phenolic content analysis. BMC Complementary Medicine and Therapies, 25, 113.

Veeraraghavan, V. P., Hussain, S., Papayya Balakrishna, J., Dhawale, L., Kullappan, M., Mallavarapu Ambrose, J., & Krishna Mohan, S. (2022). A comprehensive and critical review on ethnopharmacological importance of desert truffles: Terfezia claveryi, Terfezia boudieri, and Tirmania nivea. Food Reviews International, 38(sup1), 846–865.

Venturella, G., Ferraro, V., Cirlincione, F., & Gargano, M. L. (2021). Medicinal mushrooms: bioactive compounds, use, and clinical trials. International Journal of Molecular Sciences, 22(2), 634.

Wasser, S. P. (2002). Medicinal mushrooms as a source of antitumor and immunomodulating polysaccharides. Applied Microbiology and Biotechnology, 60, 258–274.

Wasser, S. P. (2011). Current findings, future trends, and unsolved problems in studies of medicinal mushrooms. Applied Microbiology and Biotechnology, 89, 1323–1332.

Wasser, S. P. (2017). Medicinal mushrooms in human clinical studies. Part I. Anticancer, oncoimmunological, and immunomodulatory activities: a review. International Journal of Medicinal Mushrooms, 19(4), 279–317

Mustafa SEViNDiK[1]

DOI: https://doi.org./10.52091/EVIK-2025/2-4
Érkezett: 2025 június / Elfogadva: 2025 június

A Terfezia olbiensis *etanolos kivonatának antioxidáns, kolinészteráz-gátló és antiproliferatív hatásának vizsgálata*

Kulcsszavak: *Terfezia olbiensis*, antioxidáns hatás, kolinészteráz-gátló hatás, antiproliferatív hatás, biológiai aktivitás

Absztrakt

Ebben a tanulmányban a *Terfezia olbiensis* (Tul. & C. Tul.) Sacc. gomba etanolos kivonatának antioxidáns, kolinészteráz-gátló és antiproliferatív hatását vizsgáltuk. Az antioxidáns hatást a teljes antioxidáns kapacitás (TAC), a teljes oxidatív kapacitás (TOS) és az oxidatív stressz index (OSI) paraméterekkel határoztuk meg; a TAC értéke 1,983±0,049 mmol/L, a TOS értéke 16,762±0,453 µmol/L, az OSI értéke pedig 0,846±0,043 volt. Ezenkívül a T. olbiensis acetilkolinészteráz-aktivitása 91,94±1,75 µg/mL, butirilkolinészteráz-aktivitása pedig 132,08±2,09 µg/mL értéket mutatott. Megvizsgáltuk a *T. olbiensis* antiproliferatív hatását az A549 tüdőrákos sejtvonalra, és megállapítottuk, hogy a kivonat dózisfüggő módon csökkentette a sejtek életképességét. Tanulmányunk eredményei azt mutatják, hogy a *T. olbiensis* potenciális rákellenes szerként alkalmazható a tüdőrákos sejtekben. Ezek az eredmények arra utalnak, hogy a *T. olbiensis* altal kiváltott biológiai válasz, amely pozitív hatással mutat az egészségre, fontos forrás lehet a jövőbeli terápiás alkalmazásokhoz. Ezeket a hatásokat azonban részletesebb molekuláris és *in vivo* vizsgálatokkal kell megerősíteni.

[1] Biológiai Tanszék, Mérnöki és Természettudományi Kar, Osmaniye Korkut Ata Egyetem

Mustafa SEViNDiK sevindik27@gmail.com https://orcid.org/0000-0001-7223-2220

SZALAY Anna[1]

Nemzeti szabványosítási hírek

A következő felsorolásban szereplő szabványok megvásárolhatók vagy megrendelhetők az MSZT Szabványboltban (1082 Budapest VIII., Horváth Mihály tér 1., telefon: 456-6893, telefax: 456-6841, e-mail: kiado@mszt.hu; levélcím: Budapest 9., Pf. 24, 1450), illetve elektronikus formában beszerezhetők a https://ugyintezes.mszt.hu/webaruhaz/szabvany-lista címen.

A nemzetközi/európai szabványokat bevezetjük magyar nyelven, valamint magyar nyelvű címoldallal és angol nyelvű tartalommal. A magyar nyelven bevezetett nemzetközi/európai szabványok esetén külön feltüntetjük a magyar nyelvű hozzáférést.

2025. március – 2025. május hónapban bevezetett szabványok:

07.100.30 Élelmiszer-mikrobiológia

MSZ EN ISO 10272-2:2025 EV Az élelmiszerlánc mikrobiológiája. Horizontális módszer a *Campylobacter spp.* kimutatására és számlálására. 2. rész: Telepszámlálásos módszer (ISO 10272-2:2017 + Amd 1:2023) EGYESÍTETT VÁLTOZAT – Az MSZ EN ISO 10272-2:2017 és az MSZ EN ISO 10272-2:2017/A1:2023 helyett –

MSZ EN ISO 15213-1:2023 Az élelmiszerlánc mikrobiológiája. Horizontális *módszer a Clostridium* spp. kimutatására és számlálására. 1. rész: A szulfitredukáló *Clostridium* spp. megszámlálása telepszámlálásos módszerrel (ISO 15213-1:2023) – Az MSZ ISO 15213:2006 helyett –

MSZ EN ISO 16140-7:2025 Az élelmiszerlánc mikrobiológiája. Módszervalidálás. 7. rész: A mikroorganizmusokat azonosító módszerek validálásának protokollja (ISO 16140-7:2024)

13.060 Vízminőség

MSZ EN ISO 20236:2025 Vízminőség. Az összes szerves szén (TOC), az oldott szerves szén (DOC), az összes kötött nitrogén (TNb) és az oldott kötött nitrogén (DNb) meghatározása katalitikus, magas hőmérsékletű, oxidatív égetést követően (ISO 20236:2024) – Az MSZ EN ISO 20236:2022 helyett –

67 Élelmiszeripar

67.020 Élelmiszeripari eljárások

MSZ EN 17972:2025 Élelmiszerek eredetiségvizsgálata. Élelmiszer-hitelesség és -csalás. Fogalmak, szakkifejezések és meghatározások

67.060 Gabonafélék, hüvelyesek és a belőlük származó termékek

MSZ EN 16923:2023 Élelmiszerek. A T-2 toxin és a HT-2 toxin meghatározása gabonafélékben, valamint csecsemőknek és kisgyermekeknek szánt gabonatermékekben, SPE-tisztítással és HPLC-MS/MS-sel – Az MSZ EN 16923:2017 helyett –

67.080 Gyümölcsök. Zöldségek

MSZ CWA 18149:2025 Irányelvek a mezőgazdasági és élelmiszeripari szerves hulladékok újrafeldolgozására/újrahasznosítására szolgáló extraktumok jellemzésére

67.140 Tea. Kávé. Kakaó

MSZ EN 17992:2025 Élelmiszerek eredetiségvizsgálata. A 16-O-metil-kafesztol, 16-O-metil-kahweol és származékaik összegének meghatározása pörkölt kávéban ^{1}H-qNMR-rel

MSZ EN 18003:2025 Élelmiszerek eredetiségvizsgálata. A 16-O-metil-kafesztol-tartalom meghatározása zöld és pörkölt kávéban. HPLC-módszer

[1] Magyar Szabványügyi Testület (MSZT)

67.180 Cukor. Cukortermékek. Keményítő

MSZ EN 17958:2025 Élelmiszerek eredetiségvizsgálata. A mono- (fruktóz és glükóz), di- és triszacharidok $\delta^{13}C$-értékének meghatározása mézben folyadékkromatográfiás-izotóparányos tömegspektrometriával (LC-IRMS)

67.200 Étolajok és -zsírok. Olajmagvak

MSZ EN 14105:2025 Zsír- és olajszármazékok. Zsírsav-metil-észterek (FAME). A szabad és az összes glicerintartalom, valamint a mono-, di- és trigliceridtartalom meghatározása – Az MSZ EN 14105:2021 helyett –

67.220 Fűszerek és ízesítők. Élelmiszer-adalékanyagok

MSZ EN 16466-1:2025 Élelmiszerek eredetiségvizsgálata. Az ecetsav és a víz izotópelemzése ecetben. 1. rész: Az ecetsav ^{2}H-NMR-elemzése – Az MSZ EN 16466-1:2013 helyett –

67.250 Élelmiszerekkel érintkezésbe kerülő anyagok és termékek

MSZ EN 10333:2025 Acél csomagolásra. Élelmiszerrel, emberi vagy állati fogyasztásra szánt italokkal és termékekkel való érintkezésre szánt lapos acéltermékek. Ónozott acél (fehérbádog) – Az MSZ EN 10333:2005 helyett –

MSZ EN 10334:2025 Acél csomagolásra. Élelmiszerrel, emberi vagy állati fogyasztásra szánt italokkal és termékekkel való érintkezésre szánt lapos acéltermékek. Bevonat nélküli acél (feketelemez) – Az MSZ EN 10334:2005 helyett –

MSZ EN 10335:2025 Acél csomagolásra. Élelmiszerrel, emberi vagy állati fogyasztásra szánt italokkal és termékekkel való érintkezésre szánt lapos acéltermékek. Ötvözetlen, galvanizált króm- vagy króm-oxid-bevonatos acélok – Az MSZ EN 10335:2005 helyett –

67.260 Élelmiszeripari üzemek és berendezések

MSZ EN 17677:2025 Élelmiszeripari gépek. Kézműves sütő- és tésztaipari osztógépek. Biztonsági és higiéniai követelmények

2025. március – 2025. május hónapban **helyesbített szabványok:**

07.100.30 Élelmiszer-mikrobiológia

MSZ EN ISO 16654:2025 EV Élelmiszerek és takarmányok mikrobiológiája. Horizontális módszer az *Escherichia coli* O157 kimutatására (ISO 16654:2001 + Amd 1:2017 + Amd 2:2023) EGYESÍTETT VÁLTOZAT – Az MSZ EN ISO 16654:2001, az MSZ EN ISO 16654:2001/A1:2017 és az MSZ EN ISO 16654:2001/A2:2023 helyett –

Anna SZALAY[1]

Review of National Standardization

The following Hungarian standards are commercially available at MSZT (Hungarian Standards Institution, H-1082 Budapest, Horváth Mihály tér 1., phone: +36 1 456 6893, fax: +36 1 456 6841, e-mail: kiado@mszt.hu, postal address: H-1450 Budapest 9., Pf. 24) or via website: https://ugyintezes.mszt.hu/webaruhaz/szabvany-lista.

Published national standards from March 2025 to May 2025

07.100.30 Food microbiology

MSZ EN ISO 10272-2:2025 EV Microbiology of the food chain. Horizontal method for detection and enumeration of *Campylobacter* spp. Part 2: Colony-count technique (ISO 10272-2:2017 + Amd 1:2023) CONSOLIDATED VERSION – which has withdrawn the MSZ EN ISO 10272-2:2017 and MSZ EN ISO 10272-2:2017/A1:2023 –

MSZ EN ISO 15213-1:2023 Microbiology of the food chain. Horizontal method for the detection and enumeration of *Clostridium* spp. Part 1: Enumeration of sulfite-reducing *Clostridium* spp. by colony-count technique (ISO 15213-1:2023) – which has withdrawn the MSZ ISO 15213:2006 –

MSZ EN ISO 16140-7:2025 Microbiology of the food chain. Method validation. Part 7: Protocol for the validation of identification methods of microorganisms (ISO 16140-7:2024)

13.060 Water quality

MSZ EN ISO 20236:2025 Water quality. Determination of total organic carbon (TOC), dissolved organic carbon (DOC), total bound nitrogen (TNb) and dissolved bound nitrogen (DNb) after high temperature catalytic oxidative combustion (ISO 20236:2024) – which has withdrawn the MSZ EN ISO 20236:2022 –

67 Food technology

67.020 Processes in the food industry

MSZ EN 17972:2025 Food authenticity. Food authenticity and fraud. Concepts, terms and definitions

67.060 Cereals, pulses and derived products

MSZ EN 16923:2023 Foodstuffs. Determination of T-2 toxin and HT-2 toxin in cereals and cereal products for infants and young children by SPE clean up and HPLC-MS/MS – which has withdrawn the MSZ EN 16923:2017 –

67.080 Fruits. Vegetables

MSZ CWA 18149:2025 Guidelines for characterization of extracts for the recycling/upcycling of organic agrifood wastes

67.140 Tea. Coffee. Cocoa

MSZ EN 17992:2025 Food authenticity. Determination of the sum of 16-O-Methylcafestol, 16-O-Methylkahweol and their derivatives in roasted coffee by ^{1}H-qNMR

MSZ EN 18003:2025 Food authenticity. Determination of 16-O-Methylcafestol content of green and roasted coffee. HPLC-method

67.180 Sugar. Sugar products. Starch

MSZ EN 17958:2025 Food authenticity. Determination of the δ^{13}C value of mono- (fructose and glucose), di-, and trisaccharides in honey by liquid chromatography-isotope ratio mass spectrometry (LC-IRMS)

[1] Hungarian Standards Institution

67.200 Edible oils and fats. Oilseeds

MSZ EN 14105:2025 Fat and oil derivatives. Fatty Acid Methyl Esters (FAME). Determination of free and total glycerol and mono-, di-, triglyceride contents – which has withdrawn the MSZ EN 14105:2021 –

67.220 Spices and condiments. Food additives

MSZ EN 16466-1:2025 Food authenticity. Isotopic analysis of acetic acid and water in vinegar. Part 1: ^{2}H-NMR analysis of acetic acid – which has withdrawn the MSZ EN 16466-1:2013 –

67.250 Materials and articles in contact with foodstuffs

MSZ EN 10333:2025 Steel for packaging. Flat steel products intended for use in contact with foodstuffs, products and beverages for human and animal consumption. Tin coated steel (tinplate) – which has withdrawn the MSZ EN 10333:2005 –

MSZ EN 10334:2025 Steel for packaging. Flat steel products intended for use in contact with foodstuffs, products and beverages for human and animal consumption. Non-coated steel (blackplate) – which has withdrawn the MSZ EN 10334:2005 –

MSZ EN 10335:2025 Steel for packaging. Flat steel products intended for use in contact with foodstuffs, products or beverages for human and animal consumption. Non-alloyed electrolytic chromium/chromium oxide coated steel – which has withdrawn the MSZ EN 10335:2005 –

67.260 Plants and equipment for the food industry

MSZ EN 17677:2025 Food processing machinery. Craft bakery and pastry depositors. Safety and hygiene requirements

Corrected national standards from from March 2025 to May 2025

07.100.30 Food microbiology

MSZ EN ISO 16654:2025 EV Microbiology of food and animal feeding stuffs. Horizontal method for the detection of *Escherichia coli* O157 (ISO 16654:2001 + Amd 1:2017 + Amd 2:2023) CONSOLIDATED VERSION – which has withdrawn the MSZ EN ISO 16654:2001, MSZ EN ISO 16654:2001/A1:2017 and MSZ EN ISO 16654:2001/A2:2023 –

For further information please contact Ms Anna Szalay, sector manager on food and agriculture, e-mail: a.szalay@mszt.hu

Szerzőink / Authors

Itir ERKAN
Istanbul Yeni Yuzyil University, Faculty of Health Sciences, Department of Occupational Health and Safety / Isztambuli Yeni Yuzyil Egyetem, Egészségtudományi Kar, Munkaegészségügyi és Biztonsági Tanszék

Dhanuja KALAISELVAN
Chemical Biology Lab (ASK-II-409), School of Chemical and Biotechnology, SASTRA Deemed University, Thanjavur, Tamilnadu, India / Kémiai biológia Labor (ASK-II-409), Kémiai és Biotechnológiai Kar, SASTRA Deemed Egyetem, Thanjavur, Tamilnadu, India

Judit KRISCH
Department of Food Engineering, Faculty of Engineering, University of Szeged, Szeged, Hungary / Élelmiszermérnöki Intézet, Mérnöki Kar, Szegedi Tudományegyetem, Szeged, Magyarország

Waminee NIRAMIT
Department of Food Engineering, Faculty of Engineering, University of Szeged, Szeged, Hungary / Élelmiszermérnöki Intézet, Mérnöki Kar, Szegedi Tudományegyetem, Szeged, Magyarország

Aruzhan OTEGENOVA
Department of Food Engineering, Faculty of Engineering, University of Szeged, Szeged, Hungary / Élelmiszermérnöki Intézet, Mérnöki Kar, Szegedi Tudományegyetem, Szeged, Magyarország

RAISYA
Department of Nutrition, Faculty of Sports and Health Sciences, Universitas Negeri Surabaya, Surabaya, Indonesia / Táplálkozástudományi Tanszék, Sport- és Egészségtudományi Kar, Negeri Surabaya Egyetem, Surabaya, Indonézia

Mustafa SEViNDiK
Department of Biology, Faculty of Engineering and Nature Sciences, Osmaniye Korkut Ata University / Biológiai Tanszék, Mérnöki és Természettudományi Kar, Osmaniye Korkut Ata Egyetem

Balázs P. SZABÓ
Department of Food Engineering, Faculty of Engineering, University of Szeged, Szeged, Hungary / Élelmiszermérnöki Intézet, Mérnöki Kar, Szegedi Tudományegyetem, Szeged, Magyarország

SZALAY Anna
Magyar Szabványügyi Testület (MSZT) / Hungarian Standards Institution

Vadivel VELLINGIRI
Chemical Biology Lab (ASK-II-409), School of Chemical and Biotechnology, SASTRA Deemed University, Thanjavur, Tamilnadu, India / Kémiai biológia Labor (ASK-II-409), Kémiai és Biotechnológiai Kar, SASTRA Deemed Egyetem, Thanjavur, Tamilnadu, India

Aulia Putri Srie WARDANI
Department of Nutrition, Faculty of Sports and Health Sciences, Universitas Negeri Surabaya, Surabaya, Indonesia / Táplálkozástudományi Tanszék, Sport- és Egészségtudományi Kar, Negeri Surabaya Egyetem, Surabaya, Indonézia

Élelmiszervizsgálati Közlemények / Journal of Food Investigation
Kiadó / Publisher: Szegedi Tudományegyetem, 6720 Szeged, Dugonics tér 13. / **HU ISSN 2676-8704**
Felelős kiadó: a Szegedi Tudományegyetem rektora
Főszerkesztő / Editor-in-chief: Prof. Dr. BÁNÁTI Diána
Szerkesztő / Editor: NYERGESNÉ Dr. ILLÉS Erzsébet

Borítókép / Cover image: Wikimedia Commons

Layout: Adworks Kft.

A lap negyedévente, elektronikus formában jelenik meg. / This e-journal is published quarterly.

A szakfolyóiratot a következő figyelőszolgáltatások vették jegyzékbe és referálják / this journal is listed by the following monitoring services: SCOPUS, SCIMAGO, MATARKA / *Hungarian Periodicals Table of Contents*, Magyar Tudományos Akadémia Könyvtár és Információs Központ, Magyar Tudományos Művek Tára / *Hungarian Academy of Sciences, Library of Information Centre, Hungarian Scientific Bibliography Database / Publishers International Linking Association Inc. (Crossref (DOI) Registration Agency)*