3RD INTERNATIONAL CONFERENCE ON RAW MATERIALS TO PROCESSED FOODS

18 – 19 MAY 2023

PROCEEDINGS BOOK

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RPFOODS 2023 CONFERENCE PROCEEDINGS

3rd International Conference on Raw Materials to Processed Foods

Editors

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Table of Contents

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ANGEL C. BARRACHINA-SPAIN	ASTA JUDZENTIENE-CANADA
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Conference Programme

3rd International Conference on Raw Materials to Processed Foods 2023				
Scientific Program – Oral Sessions ***Thursday 18 May 2023***				
09:00			**Welcome Reception and Registration**	
10:30	10:40		**Opening Conference** Prof. Dr. Serkan SELLI – Prof. Dr. Hasim KELEBEK	
10:45	11:30		**Opening Speech:** Targeted Prebiotic Fibers for Gut Health **Dr. BRUCE HAMAKER**	
11:30	11:45		***Concurrent Session 1*** *Session Chair: Dr. A. Aït-Kaddour*	***Concurrent Session 2*** *Session Chair: Dr. B. Kovács*
			Fireweed (*Chamerion angustifolium* (*L.)* Holub) and its biologically active organic compounds **Jurga Budiene**, Asta Judzentiene, Ausra Linkeviciute	Low-intensity ultrasonics as a tool to control the quality of meat alternatives in-real-time during processing **Filiz Koksel**, Reine-Marie Guillermic, John H. Page, James D. House
11:45	12:00		Nutritional content and health benefits of orange and purple carrot-based smoothies enriched with sour cherry and apple juices during the 3- and 6-month storage periods **Emel Hasan Yusuf**	Development of Mediterranean cereal foods (bread and bulgur) with high beta-glucan content **Zeynep Hazal Tekin Cakmak**, Hamit Koksel
12:00	12:15		Investigations of thermal treatment and extraction process on the micro-plastic profile in shrimp **Elif Tugce Aksun Tumerkan**	Impact of oat-drink residue flour on the white bread's dough properties and baking characteristics **Mahmoud Rashed**
12:15	12:30		The effect of different organic and chemical fertilizer applications on growth and seed yield of cowpea (*Vigna unguiculata L.)* **Aybegun Ton**	The use of protein hydrolysates in the enrichment of confectionery products **Zeynep Saliha Güneş,** Sebahat Şişman, İbrahim Gülseren
12:45	13:45		**Lunch**	
14:00	14:30		**Plenary Session:** Sensory evaluation of foods with geographical indication **Dr. ÁNGEL ANTONIO CARBONELL-BARRACHINA** **Luis Noguera-Artiaga, Hanán Issa-Issa**	
14:40	14:55		***Concurrent Session 3*** *Session Chair: Dr. M. Bordiga*	***Concurrent Session 4*** *Session Chair: Dr. M. Simsek*
			The effect of aging on chemical and organoleptic parameters of Monastrell wines **Reyhan Selin Uysal,** Hanan Issa-Issa, Esther Sendra, Ángel A. Carbonell-Barrachina	Investigating the antimicrobial susceptibility of raw chicken Campylobacter isolates to erythromycin and benzalkonium chloride **Dimitra P Kostoglou,** Aikaterini Kochliou, Fikirete Samo, Efstathios Giaouris
14:55	15:10		Burden of disease estimation based on *Escherichia coli* quantification from ready-to-eat meals of institutional canteens Leonor Antunes, António Lopes João, Telmo Nunes, **Ana Rita Henriques**	Designing of texture-modified fruit juices by adding different hydrocolloids **Derya Alkan**, Buse Akcay
15:10	15:25		Production of shalgam juice and its' functional properties **Tuğba Simsek**, Murat Intepe, Umit Karaaslan, Betul Kabak, Nadide Mutluer, Alp E Sahin	Enzymatic synthesis of new bioactive compounds endowed with antioxidant and emulsifying activities **Zied Zarai,** Mohamed Bouaziz, Mecit Halil Oztop
15:25	15:40		Application of anthocyanin extracts encapsulated by double emulsion method to ice cream Arzu Golge, **Meric Simsek Aslanoglu**	Pectin and gelatin-based nanocomposite biodegradable films containing sweetgum bark extract **Dilara Konuk Takma**, Hilal Şahin Nadeem
15:40	15:55		Life cycle assessment analysis of novel dryer prototype: Carbon fiber-assisted dryer **Ömer Faruk Çokgezme,** Deniz Döner, Yaren Sariduman, Eren Deniz Konak, Filiz İçier	Edible insects: Tendency or necessity for functional foods Konstantina Papastavropoulou, Marios Kostakis, Emel Oz, Fatih Oz, JianBo Xiao, **Charalampos Proestos**
			Break	
18:30	22:00		**GALA DINNER AND BOAT TOUR**	

Scientific Program – Oral Sessions

Friday 19 May 2023

09:00		**Registration**	
10:00	10:30	**Plenary Session:** Non-thermal food processing techniques, Food Industry 4.0 and Sustainability **Dr. ANET REZEK JAMBRAK**	
10:45	11:00	***Concurrent Session 1*** *Session Chair: Dr. Z. Zarai* Effects of cold plasma applications on bioactive composition of foods **Celale Kırkın**	***Concurrent Session 2*** *Session Chair: Dr. M. Bouaziz* Antimicrobial effect of cell-free supernatants of *Lactiplantibacillus plantarum* strains at different growth conditions **Ceren Ilgaz,** Nisa Olmez, Hasim Kelebek, Pınar Kadiroglu
11:00	11:10	2T2D COS PLS-DA applied to multispectral imaging to discriminate beef muscles **Abderrahmane Aït-Kaddour**, Oumayma Boukria, Jasur Safarov, Donato Andueza, Anne Listrat	Effects of preharvest hexanal treatment on chemical compositions of raspberry fruit during storage **Ayse Tulin Oz**, Duygu Ayvaz Sonmez, Ebru Kafkas
11:10	11:25	Characterization of probiotic candidate lactic acid bacteria isolated from "Dadih" a fermented buffalo milk as biopreservation in beef **Tri Yuliana,** Farah Nabilla Tyano, Vira Putri Yarlina, Putri Widyanti Harlina, Ratu Safitri, Annisa Krama	Spontaneous fermentation process of Ivorian cocoa (*Theobroma cacao L.)* beans and microorganisms involved **Kouame Fulbert Oussou**
11:25	11:40	Nutritional value assessment of *Adansonia digitata* leaves in Sudan Abdelhakam Esmaeil Mohamed Ahmed, Massimo Mozzon, **Ayaz Mukarram Shaikh,** Béla Kovács	Investigation of some chemical properties of yogurts fortified with lyophilized purslane (*Portulaca oleracea L.)* during storage **Ayse Burcu Aktas**
11:40	11:55	Fermentation of bergamot (*Citrus bergamia*) fruit with *Lactobacillus plantarum*: Phenolic compounds, antioxidant activity, and prebiotic properties Ozlem Aslan, Bora Ekinci, **Ahmet Salih Sonmezdag**	Determination of antioxidant potential, phenolic and aroma profile in *Juniperus drupacea* **Esra Ereli**, Merve Carıkcıoglu, Hasim Kelebek
12:00	13:00	**Lunch**	
13:15	13:45	**Plenary Session:** HS-GC-IMS: A "new" analytical challenge applied to food processing quality and integrity **Dr. MATTEO BORDIGA**	
13:45	14:00	***Concurrent Session 3*** *Session Chair: Dr. G. Gunes* Production of new phenolic compounds with antioxidant activities Zied Zarai, Mecit Halil Oztop, **Mohamed Bouaziz**	***Concurrent Session 4*** *Session Chair: Dr. C. Proestos* The effect of biomaterials coating on sensory properties of potatoes during storage **Hadeel Mohammad Obeidat,** Haneen Nayef Tarawneh
14:00	14:15	Characterization of volatiles and key odorants of Akpi (*Recinodendron heudoletii*) nuts as affected by single and double roasting process **Kouame Fulbert Oussou,** Serkan Selli	Green walnut extract: A novel ingredient for enhancing bee products **Ayaz Mukarram Mr Shaikh**, Béla Kovács, Abdelhakam Esmaeil Mohamed Ahmed, Csaba Oláh, Lajos Daróczi, Hassan El-Ramady, József Prokisch
14:15	14:30	Changes in biochemical compositions of blueberry fruit during shelf life **Ayse Tulin Oz,** Betul Yesil, Ebru Kafkas	Survival of *Listeria monocytogenes* in date palm paste and syrup at different storage temperatures **Murad A. Al-Holy,** Amin N. Olaimat, Mahmoud H. Abu Ghoush
14:30	14:45	Phenolic compound profiles coupled with Chemometrics as a tool for authentication of Albanian wines **Dritan Topi**, Ardiana Topi, Gamze Guclu, Serkan Selli, Turkan Uzlasir, Hasim Kelebek	Pistachio hull extract as a microbial control agent against food pathogens **Gamze Seker,** Meltem Yesilcimen Akbas
14:45	15:00	The characterization of licorice molasse's bioactive compounds and the determination of antioxidant properties **Onur Sevindik**	Physicochemical, pasting, and thermal properties of water chestnut starch – xanthan gum complexes as influenced by the addition of sucrose at different concentrations **Zubala Yasir,** Qudsiyah Kalim

15:00	15:15	**Break**		
		Concurrent Session 5 *Session Chair: Dr. D. Topi*	***Concurrent Session 6*** *Session Chair: Dr. Z. Yasir*	***Concurrent Session 7*** *Session Chair: Dr. A.S. Sonmezdag*
15:15	15:30	The effect of heat moisture treated-banana flour addition as composite material of noodle on its post prandial glucose profile and noodle characteristics **Yana Cahyana**, Tien Siti Halimah, Herlina Marta	Effect of different *Lactobacillus* strains on a fermented beverage from chickpea and date **Nisa Ölmez,** Ceren Ilgaz, Pınar Kadiroglu	A comparative study on phytochemical evaluation of *Citrus auriantum* and *Citrus paradisi* juices **Ozge Aksay,** Serkan Selli, Hasim Kelebek
15:30	15:45	Effect of dual-modification by heat-moisture treatment and octenyl succinic anhydride (OSA) on physicochemical and emulsion properties of arrowroot (*Maranta arundinaceae* L.) starch **Herlina Marta**, Ari Rismawati, Yana Cahyana, Mohamad Djali	Investigation of antimicrobialeffect of hazelnut green huskethanolic extract **Pelin Kiraz**, Meltem Yeşilçimen Akbaş	Detection of off-odorants in food matrices by the application of molecularly imprinted polymers **Nurten Cengiz,** Hasim Kelebek, Serkan Selli
15:45	16:00	Microbial evaluation of fermented beetroot juice produced by probiotic *Lacticaseibacillus paracasei* Gamze Durukan, Ferda Sari, **Hatice Aybuke Karaoglan**	Bioactive composition and antioxidant activity of *Spirulina platensis* **Turkan Uzlasır,** Serkan Selli, Hasim Kelebek	Aroma components of *Glycyrrhiza glabra* molasses **Melike Dagli,** Gamze Guclu, Pinar Kadiroğlu Kelebek, Hasim Kelebek, Serkan Selli
16:00	16:15	Development of a fermented plant-based product structure from pistachio **Erenay Erem,** Meral Kilic-Akyilmaz	Comparative evaluation of bioactive compounds changes from white to black garlic **Hatice Kubra Sasmaz,** Hasim Kelebek	Characterization of biodegradablefilms prepared from chemically modified pearl millet starches **Marium Shaikh**, Tahira Mohsin Ali
16:15	16:30	Determination of vinegar adulteration using stable carbon isotope analyzer **Onur Sevindik,** Gamze Guclu, Hasim Kelebek, Serkan Selli	Amino acid and fatty acid changesin ostrich meat by treating gamma irradiation and kale leaf powder **Muhammad Sajid Arshad,** Waseem Khalid	Effect of pH and brewing methods on volatile nitrogen compounds in Turkish coffee **Fırat Can,** Kemal Sen
16:30	16:45	The value of the waste products of date fruit (*Phoenix dactylifera)* **Afaf Kamal-Eldin**, Ali Al Marzouqi, Mutamed Ayyash	Investigation of veterinary drug residues in available meat in muscat, Sultanate of Oman **Sumaiya Al Kindi**, Alka Ahuja, Razna Al Maimani, Mohammed Al Balushi, Ahlam Al Kharusi	Assessing antimicrobial resistancein *E. coli* isolated from salad vegetables in UAE: Phenotypic and genomic characterization **Ihab Habib**, Rami H Al-Rifai, Mohamed-Yousif Ibrahim Mohamed, Akela Ghazawi, Afra Abdalla, Glindya Lakshami, Neveen Agamy, Mushtaq Khan
16:45	17:00	Development of experimental equipment for vegetables and fruits Panasenko A.S., **Safarov J.E.**	Energy efficient technology for drying food Safarov J.E., **Panasenko A.S.,** Sultanova Sh.A.	Storing vegetables and fruits using ultraviolet light Safarov J.E., Pulatov M.M., **Sultanova Sh.A.**
17:00	17:15	Microwave-assisted drying of mango peels: Drying kinetics and optimization of process conditions usingmathematical models and response surface methodology **Srutee Rout**, Prem Prakash Srivastav	Knowledge, attitudes and dietary practices of health professionals regarding sustainable diet **Nevena Ivanović**, Milica Zeković, Jelena Kukić Marković, Margarita Dodevska, Milan Jovanovic Batut, BrižitaDjordjević	Comparative study on functional characteristics of commercial palm shortening and oleogels prepared from high oleic sunflower oil Hamza Ismail, **Tahira Mohsin Ali**, Natasha Abbas Butt
		Closing Remarks Prof. Dr. Serkan SELLI – Prof. Dr. Hasim KELEBEK		

International Conference on Raw Materials to Processed Foods 2023	
Scientific Program – Poster Sessions *Thursday 18 May 2023*	
	Method of storing agricultural products **Pulatov M.M.,** Safarov J.E.
	Niosomes as nanocarriers for encapsulation of food ingredients **Busra Alper,** Pınar Kadiroglu
	Food safety and hygiene knowledge, attitudes, and practices assessment of industrial food handlersRita Côrte-Real, **Ana Rita Henriques**
	Exploring mycelium as a sustainable and alternate protein source for developing iron and vitamin D2 richlow moisture meat analogue **Shubham Mandliya**, Siddharth Vishwakarma, Hari Niwas Mishra
	Near-Infrared spectroscopy quality parameter analysis in wheat from Albania Lorena Mato, Ardiana Topi, Arben Osmanaj, Bujar Neziraj, Bekri Xhemali, Milot Tufaj, **Dritan Topi**
	Antimicrobial, antioxidant, and phytochemical properties of *Citrus maxima* (pomelo) peel extracts **Gamze Seker**, Meltem Yesilcimen Akbas
	Nutritional composition and fatty acid profile of red goji berry (*Lycium barbarum*) cultivated in Serbia **Tijana D Ilić,** Ivana D Djuricic, Bojana Vidović
	The anti-biofilm effect of hazelnut green husk ethanolic extract **Pelin Kiraz**, Meltem Yeşilçimen Akbaş
	Prototype unit for continuous manufacturing of milk tablets **Pruthiraj Hial**, Siddharth Vishwakarma, Hari Niwas Mishra
	Atmospheric cold plasma application on tomatoes **Omer Faruk Cokgezme**
	Investigation of the antifungal activity of postbiotics **Zeynep Akinan**, Dilara Nur Dikmetas, Funda Karbancıoglu-Guler
	An alternative biological control method against *Aspergillus carbonarius* growth **Guliz Konusur**, Dilara Nur Dikmetas, Hatice Funda Karbancıoglu Guler
	Encapsulation of aqueous *Hibiscus sabdariffa* extract in high internal phase emulsions stabilized by soy protein isolate **Hümeyra Cavdar**, Esra Capanoglu Guven
	Probiotic viability during the shelf life of a novel Greek sheep traditional yogurt and following subsequentin vitro digestion Ioanna Gkitsaki, Panagiota Potsaki, Ioanna Dimou, Zoi Laskari, **Dimitra P Kostoglou**, Antonios Koutelidakis, Efstathios Giaouris

The effect of herb addition to the antimicrobial properties of Turkish black tea **Berfu Gelen**
Bioactive properties of *Pistachia lentiscus* leaves and fruits **Ozlem Kilic Buyukkurt**
Biochemical composition and health effects of *Phaeodactylum tricornutum* **Turkan Uzlasir**
Synthetic hexaploid wheats: phenolic acid composition and antioxidant capacity **Zeynep Hazal Tekin Cakmak,** Hamit Koksel
Determination of differences in sulfur compound composition of fresh and black garlic samples **Hatice Kubra Sasmaz**
Antioxidant and metal chelating activities of Dandelion (*Taraxacum officinale* (G.H. Weber ex Wiggers)leaves **Fadime Eryilmaz Pehlivan**
Free radical scavenging and metal chelating activities of *Beta vulgaris* subsp. *maritima* (L.) Arcang **Fadime Eryilmaz Pehlivan**
Light spectral modulation of phenolic synthesis in *Ocimum basilicum* L. **Andrei Lobiuc**, Naomi Paval, Marian Burducea, Vasile Stoleru, Maria-Emiliana Fortuna
Conjugation of polycaprolactone for reduced siloxane impact towards crop and microbial species **Maria-Emiliana Fortuna**, Andrei Lobiuc, Elena Ungureanu, Valeria Harabagiu
A simple electrochemical method for nickel detection in vegetables and fruits **Liliana Anchidin-Norocel**
Sugar profile of seed obtained from abbas fig variety and fatty acid content of seed oil **Tulin Eker,** Ayse Tulin Oz
Physicochemical characteristics of aquafaba and applications in food industry **Dilek Kaya Sarigül**, Sinem Ece Bekdemir, Nilay Sekerin
Fingerprint of key odorants, fatty acids profiles, and antioxidant potential of Akpi (*Recinodendronheudoletii*) nuts as influence roasting process **Kouame Fulbert iu**
The effects of using chia and flaxseed as egg replacements on the quality of the cakes **Derya Alkan**, Neval Burkay

Enzymatic synthesis of new bioactive compounds endowed with antioxidant and emulsifying activities

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ABSTRACT

Biomolecules such as polyphenols and sugars are highly polar in nature owing to the abundance of hydroxyl moieties. Although these are employed for several applications in foods, their compromised lipophilicity poses some limitations therein. One of the approaches to introduce lipophilicity in polyphenols and sugars, and consequently their functional properties, is through acylation. This has emerged as an area of extensive research in recent times. The current work highlights the significance of esterification of polyphenols and sugars and the effect of this reaction on physicochemical properties of polyphenols and sugars.

In the present study, the lipase-catalyzed synthesis of Hydroxytyrosol fatty acid esters from oleuropein and fatty acids and in the presence of ethyl acetate was investigated. The influence of different experimental parameters such as the loading of lipase, the reaction duration or the use of a co-solvent was studied and the reaction conditions were optimized with Hydroxytyrosol. Moreover, it was possible to determine the site where the reaction occurred, indeed the hydroxyl group which reacted with the fatty acid is the least chemically hindered and the most available which of the linear chain (-CH2-CH2- OH).

This study presents the essential enzymatic modifications that led to the synthesis of bioactive compounds with attractive emulsifying properties for the food industry by emphasizing on optimization of the reaction conditions to maximize the production yields. Lastly, the identification and the characterization have been studied for the potential food applications.

Keywords: Lipase, phenolic compounds, sugars, lipophilization, emulsions

Production of new phenolic compounds with antioxidant activities

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ABSTRACT

The oxidation of unsaturated lipids, which is one of the major causes of foods adulteration, may be limited by the use of antioxidants. Nowadays, phenolic compounds (particularly phenolic alcohol, acids and flavonoids) generate a growing interest owing to their antioxidant and emulsifying properties. However, the implementation of such polar molecules in lipid-based systems (emulsion or other) is difficult and can lead to a decrease of their efficiency. To solve this issue, one strategy consists in adjusting the polarity of these molecules by the grafting of aliphatic chains of different lengths. The first part of this work has been dedicated to the enzymatic synthesis of a series of Oleuropein, Tyrosol and hydroxytyrosol alkyl esters. The best results were obtained using a two-steps strategy. This strategy involves a preliminary hydrolysis with lipase in presence followed by an enzymatic synthesis with Novozyme of tyrosol and Hydroxytyrosol, with acids of 6-18 carbon chain lengths. Under optimal conditions, the initial rates of esterification were up to two-fold higher with Novozyme than that of other used enzymes. In the second part of this work, we evaluated, from kinetic and stationary point of view, the impact of obtained compounds on the ability of new molecules to scavenge the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH). The results showed that the increase of alkyl chain length did not necessarily lead to an improvement in the antioxidant capacity. Indeed, maximum antioxidant capacities for chain length of 12 and 8 carbon atoms were observed for fatty esters of Tyrosol and hydroxytyrosol, respectively.

Keywords— Lipase, Novozyme, phenolic compounds, lipophilization, antioxidant, emulsions.

Pistachio hull extract as a microbial control agent against food pathogens

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ABSTRACT

Pistacia vera (L.) is a valuable agricultural product that is produced in high amounts in Turkey. Wastes of pistachio such as hulls can have potential bioactivity on procaryotic and eucaryotic cells. The use of pistachio hull as a food preservative has been investigated by researchers (Aliyari et al., 2019). Therefore, it is important to determine its activity against food-borne pathogenic bacteria. In addition, the pistachio hull is in rich phytochemicals, indicating its potential use as a food preservative in the food industry.

In this study, antimicrobial activities of methanolic extract of pistachio hull against some food pathogens (*Escherichia coli* ATCC 25922, Enterobacter aerogenes ATCC 13048, Enterococcus faecalis ATCC 29212, Staphylococcus aureus ATCC 25923, Staphylococcus aureus ATCC 29213, Pseudomonas auroginosa ATCC 27853, Streptococcus uberis ATCC 700407, *Bacillus cereus* ATCC 11778 and *Bacillus subtilis* ATCC 6633) that cause critical problems in food processing industry were tested (Molina et al., 2020). Inhibitory activities of methanolic extract of pistachio hull against quorum sensing providing communication in bacterial communities for biofilm formation was evaluated by using *Chromobacterium violaceum* strains. The inhibitory activity of pistachio hull methanolic extract on bacterial movement (anti-swarming activity: 100%) was also tested against Pseudomonas auroginosa PA01. In addition, antioxidant activity (DPPH radical scavenging activity) and phytochemical contents (gallic acid:3675±309 µg/mL; quercetin: 33±1.8µg/mL; total phenolic contents (TPC): 48981±2780 µg/mL gallic acid equivalent; total flavonoid contents (TFC): 2251±369 µg/mL quercetin equivalent) were determined. As a result, it was shown that the methanolic extract of pistachio hull can be used as an alternative natural bioactive agent in the food industry.

Keywords: Antibiofilm, antimicrobial, anti-quorum sensing, pistachio hull methanolic extract antimicrobial.

Survival of *listeria monocytogenes* in date palm paste and syrup at different storage temperatures

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ABSTRACT

Contamination of low water activity foods including palm date with pathogenic bacteria is a major concern worldwide. The objective of the current study was to investigate the behavior of *Listeria monocytogenes* in processed date paste and syrup at different temperatures. Processed date paste and syrup were inoculated with approximately 6.6 log CFU/ml of cocktail cultures of five strains of *L. monocytogenes* and stored at 4, 10 and 24°C for 90 d.. *L. monocytogenes* numbers decreased by 1.4, 4.4 and >4.6 log CFU/g in date paste stored at 4, 10 and 24 °C, respectively. In date syrup, numbers of *L. monocytogenes* decreased to undetectable levels (>4.6 log CFU/g reduction) by 50, 14 and 4 d at 4, 10 and 24 °C, respectively by direct plating method and a complete inhibition was observed at 10 and 24 by 50 and 30 d of storage, respectively. The initial pH values of date paste and syrup were 4.7 and 4.8, respectively; and remained stable until the end of storage period except for *L. monocytogenes*-inoculated syrup, where the pH has dropped significantly. *L. monocytogenes* can survive well in date products particularly at refrigerator temperature, which underscores the necessity of preventing the contamination of date products to reduce the potential risk associated with foodborne pathogens.

Keywords: Low water activity, Salmonella, Date paste, Date syrup, Growth behavior

Amino acid and fatty acid changes in ostrich meat by treating gamma irradiation and kale leaf powder

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ABSTRACT

The current study was carried out to measure the effect of gamma irradiation and kale leaf powder on amino and fatty acids profile of ostrich meat at different storage intervals. Gamma irradiation (3 kGy) with or without kale leaf powder (1 % and 2 %) was applied. The significant changes ($p \leq 0.05$) in outcomes were observed on different treatments (kale leaf powder and irradiation) and storage intervals. The pH value of the ostrich meat sample decreased with the addition of kale leaf powder whereas, the increment was found on irradiation with the passage of time. The minimum reduction in amino acids and fatty acid profile in ostrich meat samples were measured after being irradiated dose during different storage intervals. However, with the addition of kale leaf powder (KLP), the value of amino acids and fatty acids in ostrich meat samples was improved. Conclusively, the pH was observed to be reduced on combined treatment (irradiation + KLP) whereas, the 2% KLP improved the amino acids and fatty acid profile of ostrich samples.

Phenolic compound profiles coupled with chemometrics as a tool for authentication of Albanian wines

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ABSTRACT

High interest is present in studying wines produced in Albania due to grape cultivars' diversity and scarce data. Phenolic compound profiles of red and white wines from five *Vitis vinifera* grape cultivars*: Kallmet, Shesh i zi, Shesh i bardhë, Merlot, and Cerruje,* are presented. Thirty-one phenolic compounds belonging to sub-groups: flavonoids and non-flavonoids, were identified and quantified by Liquid Chromatography coupled with Tandem Mass-spectrometry. Territory and vintage, were evaluated and compared in this investigation. Among red wines, *Shesh i zi* cv. presented the highest phenolic content (1037.53 mg L-1), followed by *Kallmet* cv. (539.62 mg L-1), while in the white wines group, those from *Shesh i bardhë* cv were distinguished (699.78 mg L-1). The main phenolic in the studied wines was found to be gallic acid. Among flavanols, (+)-catechin was found in the highest levels, reaching the maximum to Kallmet red wine (58.91 mg L-1), followed by (-)-epicatechin (29.15 mg L-1). Procyanidin dimers presented by procyanidin B3 to highest levels in grape cultivars belonging to *Shesh i bardhe* (215.23 mg L-1) and *Shesh i zi* (136.30 mg L-1). *Merlot* wines revealed the highest content of flavonols with quercetin-3-O-glucuronide and quercetin-3-O-glucoside. The highest quantity of stilbenoids belonged to *Kallmet* red wines (1.59 mg L-1). Authentication of wines through PCA indicates that these wines are easily distinguished according to grape cv. and region. At the same time, there is not enough evidence to conclude according to the vintage.

Phenolic profiles coupled to chemometrics as a tool for authentication of Albanian wines

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ABSTRACT

Studying wines produced in Albania due to grape cultivars' diversity and scarce data has shown high interest recently. Phenolic compound profiles from five *Vitis vinifera* grape cultivars: *Kallmet*, *Shesh i zi*, *Shesh i bardhë*, *Merlot*, and *Cerruje*, are presented in this study. Thirty-one phenolic compounds belonging to sub-groups: flavonoids and non-flavonoids, were identified and quantified by Liquid Chromatography coupled with Tandem Mass-Spectrometry. Territory and vintage influences were evaluated and compared in this investigation. Among red wines, *Shesh i zi* cv. presented the highest phenolic content (1037.53 mg/L), followed by *Kallmet* cv. (539.62 mg/L), while in the white wines, those from *Shesh i bardhë* cv. were distinguished (699.78 mg/L). Gallic acid appeared to be the main phenolic compound in the studied wines; among flavanols, (+)-catechin was found in the highest levels, with the maximum to *Kallmet cv.* red wine (58.91 mg/L), followed by (-)-epicatechin (29.15 mg/L). Procyanidin dimers presented by procyanidin B3 to the highest levels belonged to *Shesh i bardhë* (215.23 mg/L) and *Shesh i zi* (136.30 mg/L). *Merlot* wines revealed the highest content of flavonols with quercetin-3-O-glucuronide and quercetin-3-O-glucoside. The highest quantity of stilbenoids belonged to *Kallmet* red wines (1.59 mg/L). Comparing the wines originating from the same grape cultivar and vintage but different wine zones revealed their differences in phenolic compound profiles and quantities. Authentication of wines through Principal Component Analysis (PCA) indicates that these wines were easily distinguished according to grape cultivar and region. At the same time, there is not enough evidence to conclude according to the vintage.

Keywords*: Wine phenolics, Shesh i zi, Kallmet, Shesh i bardhë,* Albania

INTRODUCTION

Wine, an alcoholic beverage of fermented grape juice, is a product of high commercial value and relevant cultural aspects. Several factors, such as grape cultivar, terroir, viticultural practices, winemaking techniques, and aging conditions, impact wine quality (Merkytė, Longo, Windisch, Boselli, 2020). Economically, the grapevine (*Vitis*) is considered one of the world's major fruit crops according to the planted area (Torregrosa et al., 2015). Chemically, wine is a complex mixture of water, sugar, and organic acids, considered the main constituents. Other minor constituents, such as phenolic compounds, are key factors in the wine's characteristics (Butnariu & Butu, 2019).

Phenolic compounds are secondary metabolites in grape berries chemically transformed during winemaking (Tzachristas, Pasvanka, Calokerinos & Proestos, 2020). They play several essential functions in wine, affecting bitterness and astringency taste, especially in red wines. In addition, wine phenolics derive from wood containers during the winemaking process. They include non-flavonoids: hydroxycinamates, phenolic acids, stilbenes, and volatile phenols; and flavonoids: anthocyanins, flavan-3-ols, flavonols, etc. Anthocyanins are the main compounds in young red wines, responsible for their intense red color. During aging and maturation, due to several chemical reactions, the wines' color changes from red-violet to red-orange (de Freitas et al., 2017). Apart from genetic and environmental parameters, implementing specific oenological and storage practices deeply affects the content and nature of the polyphenols in wine (Tzachristas et al., 2020).

Flavan-3-ols, found in grape seeds, stems, and skins, structurally exist as monomers, oligomers, and polymers. Main monomers are (+)-catechin and (–)-epicatechin, while oligomers and polymers are condensed tannins or proanthocyanidins (Terrier, Poncet-Legrand & Cheynier. 2009; Jordao & Ricardo-da-Silva, 2019). They are present in numerous fruits, flowers, plant seeds, and processed foods like wine, chocolates, etc. (Qi

et al., 2022). Proanthocyanidins play an essential role in red wine's sensorial properties by contributing to the wine color and mouthfeel properties (José Gómez-Míguez, 2007; Jordao & Ricardo-da-Silva, 2019). Together with anthocyanins, flavan-3-ols are involved in the copigmentation processes contributing to the specific color of wines (He et al., 2012). Polymeric proanthocyanidins dominate (60-80%) over oligomeric forms (15-30%), and monomer flavan-3-ols are less than 10% of the total proanthocyanidins (Sun, Ricardo-da-Silva & Spranger, 2001). Their beneficial human health includes anti-inflammatory and immune regulatory functions, hypoglycaemic and hypolipidemic effects, metabolic regulation, and anticancer effects (Ma & Zhang, 2017; Qi et al., 2022).

Hydroxybenzoic and hydroxycinnamic acids in wine occur in their free or esterified forms, such as caftaric acid (trans-caffeoyl tartaric acid) or fertaric acid and coutaric acid. They are a diverse group of compounds with various health-promoting properties important for many wine sensory attributes (Vecchio et al., 2017). Non-esterified structures originate during wine fermentation as hydrolysis products. They can be oxidation substrates and precursors of the browning to white wines and may produce a bitter taste (Merkytė, Longo, Windisch, & Boselli, 2020). Many reports show the wine phenolics' health-protective properties on cardiovascular diseases, cancer, obesity, neurodegenerative diseases, diabetes, allergies, and osteoporosis (Fernandes et al., 2017).

Phenolic compounds, among them: phenolic acids, flavonoids, tannins, and stilbenes, are mainly applied for wine quality and authenticity assessment studies (Tzachristas, Pasvanka, Calokerinos & Proestos, 2020). Wine authenticity and commercial value often link to grape variety, geographical origin, and vintage year. Certain regions or countries are known for producing superior wines of higher retail value.

Wine is an easily adulterated product; consequently, the authenticity of wine contributes to consumer protection and defends the producers from unfair competition. It is a crucial issue in food quality control (Arvanitoyannis, 2010). Most authentication studies apply the well-known unsupervised exploratory principal components analysis (PCA) and/or cluster analysis techniques. The main discriminant techniques are linear discriminant analysis (LDA), k-nearest neighbor (KNN), and partial least squares-discriminant analysis (PLS-DA) (Kamiloglu, 2018).

The geographical position and relief have depicted the pedoclimatic conditions of Albania by splitting the territory into three wine-growing zones according to the EU viticulture classification (Figure 1). North-South orientated, the first wine zone includes the lowland and coastal areas. Toward the East lies the central areas of the second wine zone, followed by the eastern regions, the third wine zone (VWS, 2019). Albania's wine-growing regions may classify into Zone C-III, the warmest, split into two sub-zones, C IIIa and C IIIb, and Zone C II comprising the Balkan peninsula countries. Sub-zone C-IIIa includes the country's coastal and low-hilly western regions, reaching an elevation up to 400 m a.s.l. Sub-zone C-IIIb includes hilly pre-mountainous areas in the distance from the sea and elevations 400-800 m a.s.l. Zone C-II presents mountainous areas and plains aligned in the Eastern regions with elevations over 800 m (EU, 2009).

According to the updated Köppen and Geiger climate classification system, the country's climate is classified mainly into *Csa* and *Cfa* categories, and several regions with microclimate conditions concerning the altitude and distances from the sea (Kottek et al., 2006; Beck, 2018). This study analyzed Albanian wines from local grape cultivars Kallmet, *Shesh i zi*, *Shesh i bardhë*, *Cerruje*, and the international *Merlot* cultivar, belonging to the 2019 and 2020 vintages. Wine regions belonged to the coastal zone, including Durrësi, Tirana, and Lezha, and the inland regions Mirdita-Mati (Figure 1). This organization overlayed both wine region classifications, according to EU, 491/2009, and climate regions.

Figure 1. Wine regions according to the VWS and wine samples' origin. (Source: www.wineandvinesearch.com)

Durrësi and Kavaja regions are across the Adriatic Sea, with vineyards in hilly locations up to 200 m a.s.l. Both Shesh grape cvs. initially originating in Central Albania, nowadays dominate the coastal and inland regions. Kallmeti grape cv. is primarily present in Northwest Albania, with Shkodra and Lezha covering the main vineyards. *Cerruje* grape cultivars is present in the inland regions of Northern Albania. Climatic indicators among them are presented in Table 1. Coastal regions are exposed to above 2500 sunny hours per year, with a generally mild warm and temperate climate. Inland areas that belong to the rest of the country have an interval of 2000-2500 sunny hours per year: the long-term weather variables, temperature, precipitation, and sunny hours (1991–2021).

Table 1. Long-term weather variables for wine terroirs in the study.

County	Köppen and Geiger classification	Average annual temperatures (°C)	Average annual precipitation (mm)	Annual sunny hours (h)
Durrës	Csa	15.9	1245	3491.5
Kavaja	Csa	15.7	1245	3489.9
Tirana	Csa	14.8	1136	3447.4
Lezhë	Csa	14.6	1288	3435.8
Mirdita	Cfa	13.2	1338	3163.7
Mati	Cfa	12.2	1484	3122.1

This study aims to present the phenolic compounds' composition of mono-cultivar wines produced in Albania. Comparison of phenolic profiles among them will contribute to the efforts to authenticate red and white wines produced by native cultivars*: Kallmet, Shesh i zi, Shesh i bardhë, Cerruje*, and the international grape cultivars, *Merlot*.

2. MATERIALS AND METHODS

2.1. Chemicals

HPLC-grade methanol, acetonitrile, and formic acid (Merck, Darmstadt, Germany) were used after filtration through a 0.45-µm pore-size membrane. Chemical standards procyanidin B1, B2, B3, B4, resveratrol, and protocatechuic acid were purchased from Extrasynthese (Genay, France). Caffeic acid, caftaric acid, coutaric acid, (+)-catechin and (–)-epicatechin, fertaric acid, gallic acid, gallocatechin, isorhamnetin-3-O-glucoside, kaempferol-3-O-glucoside, *p*-coumaric acid, quercetin, quercetin-3-O-glucoside and quercetin-3-O–galactoside standards purchased by Sigma Chemical Co. (St. Louis, MO, USA).

2.2. Wine samples

Wine samples were supplied by wineries from 2019 and 2020 vintages from the administrative regions of Dibra, Durrësi, Lezha, and Tirana. According to Köppen and Geiger climate classification system Durrësi, Lezha, and Tirana regions are classified as *Csa*, while Mati and Mirdita counties, part of the study, belongs to the *Cfa* category (Kottek et al., 2006; Beck et al., 2018).

Table 2. Wine samples according to administrative and county region.

Grape cultivars	Region	County
Shesh i zi	Tirana	Kavaja
Shesh i bardhë	Tirana	Kavaja
Kallmet	Lezha	Lezha
	Lezha	Mirdita
Merlot	Tirana	Kavaja
	Dibra	Mati
Cerruje	Dibra	Mati

2.3. Liquid Chromatography-Tandem Mass-Spectrometry Analysis

High-Performance Liquid Chromatography equipment (Agilent 1260 HPLC; Agilent Tech., Palo Alto, California, USA) coupled with a diode array detector (G1351D 1260 DAD VL) was used to conduct analysis. The analytical method developed by Kelebek and coauthors employing LC-DAD-ESI-MS/MS with negative ionization mode (Table 3) was applied to analyze the phenolic compounds (Kelebek et al., 2020a; Kelebek et al., 2020b). Previously wine samples were filtered with a filter membrane of 0.45-µm pore-size and injected into the LC system. The system consisted of a binary pump (G1312 B, 1260 Bin pump), a degasser (G1322 A, 1260 Degasser), and an auto-sampler (G1367 E, 1260 HIP ALS). The Phenomenex Luna reversed-phase C-18 column with the specification of 4.6 x 250 mm and 5 µm (Torrance, California, USA) was employed in the analyses. Two mobile phases were utilized as solvent A, water/formic acid (99:1 v/v), and solvent B, acetonitrile/solvent A (60:40 v/v). Standard curves were obtained using the commercial standards at concentrations that generally exist in extracts (nearly 1–100 mg/L) and getting regression values (r2) greater

than 0.995. In the case of the reference compound absence, the calibration of similar substances was employed by considering the molecular weight correction factor. The limits of quantification (LOQ) and limits of detection (LOD) were computed by utilizing the S/N ratio values (signal-to-noise) of 10 and 3, respectively.

Table 3. Method specifications for wine phenolics identification with LC-ESI-MS/MS (negative mode).

Peak	Compounds	Abbreviation	t_R (min)	UV λ_{max} (nm)	$[M–H]^-$ (m/z)	MS/MS (m/z)
	Hydroxybenzoic acids and flavanols					
1	Gallic acid	GA	14.13	276	169	125
2	3-O-galloyl quinic acid	3-G_Qui_A	14.71	274	343	191, 169, 125
3	Protocatechuic acid-O-hexoside	PC_A-hex	17.25	296	315	153
4	Gallocatechin	Gcat	18.37	274	305	179, 125
5	Protocatechuic acid	PC_A	20.55	294	153	109
6	Epigallocatechin	EpiGCat	25.10	274	305	179, 125
7	Procyanidin B3	B3	23.64	279	577	559, 425, 289
8	Procyanidin B1	B1	29.53	279	577	559, 425, 289
9	Catechin	Cat	30.97	280	289	245, 175
10	Procyanidin B2	B2	33.86	280	577	559, 425, 289
11	Epicatechin	Ecat	37.56	280	289	245, 175
12	Procyanidin B4	B4	42.93	280	577	559, 425, 289
	Phenolic acids					
14	2-S-glutathionyl-caffeoyl-tartaric acid	2-S-glt_CaTa_A	18.89	330	616	484, 440, 272
15	*cis*-Caftaric acid	c-Caf_A	21.94	328	311	179, 149, 135
16	*trans*-Caftaric acid	t-Caf_A	24.18	328	311	179, 149, 135
17	*cis*-Coutaric acid	c-Cou_A	31.11	310	295	163, 149
18	*trans*-Coutaric acid	t-Cou_A	32.70	314	295	163
19	*cis*-Fertaric acid	c-Fer_A	34.83	322	325	193, 149
20	*trans*-Caffeic acid	t-Caf_A	35.83	323	179	135
21	*trans*-Fertaric acid	t-Fer_A	36.16	328	325	193, 149
22	p-Coumaric acid	p-Cou_A	45.94	310	163	119
	Flavonols					
23	Quercetin-3-O-galactoside	Que-3-gal	47.80	360	463	301
24	Quercetin-3-O-glucoside	Que-3-glu	48.14	360	463	397, 301
25	Quercetin-3-O-glucuronide	Que-3-glcn	48.49	355	477	301, 133
26	Isorhamnetin-3-O-glucoside	Iso-3-glu	52.32	356	477	315, 301, 300, 299
27	Quercetin	Que	63.38	355	301	151
	Stilbenoids					
28	*cis*-Piceid	c-Pic	47.46		389	227
29	*trans*-Piceid	t-Pic	53.29		389	227
30	*cis*-Resveratrol	c-Res	59.4		227	185, 159
31	*trans*-Resveratrol	t-Res	64.34		227	185, 159

2.3. Statistical analysis

Chemometric analysis was conducted by XLSTAT 2016.02.28451. Principal Component Analysis (PCA) according to vintage and wine regions for wines was conducted.

3. RESULTS AND DISCUSSIONS

3.1. Wine regions of Albania

The relief, mainly mountainous in two-thirds of the total area, has greatly influenced grape cultivation in Albania. Vine cultivation covers an area of approximately 11 thousand ha (INSTAT, 2022). It is organized

into six wine regions: Shkodra-Lezha, Central Albania, Myzeqe, Shkumbini and Devolli River valleys, Ionian Coast, and South-eastern Albania. Even though being a Mediterranean country, a diversity of micro-climate zones cover the territory. According to the Koppen-Geiger classification, the country's leading wine regions are present in the coastal zone, the *Csa* category.

In contrast, the *Cfa* climate zone is situated inland regions across river valleys (Figure 3a). Even though the Shkodra-Lezha and Central Albania regions do not have a significant size, they are distinguished for the presence of native grape cultivars vineyards. This study analyzed red wines produced by: *Shesh i zi*, Kallmet, and *Merlot* grape cultivars and white wines produced by *Shesh i bardhë* and *Cerruje* grape cultivars from these two regions, which according to VWS, comprise two climatic wine zones.

3.2. Total phenolic compounds in red and white wines

Total phenolic compounds (TPC) were quantified through the Liquid Chromatography-Tandem Mass-Spectrometry Analysis in wines of different grape cultivars: Kallmet, *Shesh i zi*, *Shesh i bardhë*, *Cerruje*, and *Merlot*. Factors like vintage and terroir are analyzed. Different vintages, 2019 and 2020, and wine regions concerning climate specificity are studied and discussed. The Kallmet grape cultivar is indigenous to Northwest Albania. Kallmet red wines were studied according to Terroir, Lezha, and Mirdita, and 2019 and 2020 vintages that belong to *Csa* and *Cfa* climatic zones were analyzed, respectively. Wine samples from the Mirdita region reveal a TPC of 380.10 mg/L, much lower than wines from the Lezha region, 500.83 mg/L, for both vintages. Higher TPC amount in wine samples to the 2020 vintage, 462.41 mg/L, compared with 539.26 ± 3.24 mg/L of the 2019 vintage, in the case of the Lezha region.

Shesh i zi grape cultivar indigenous to Central Albania, specifically, Tirana district. **The Tirana district includes Tirana and Kavaja counties**, which belong to *Csa* climatic zone. *Shesh i zi* red wines revealed TPC values (939.31 mg/L) much higher than Kallmet wines, 440.47 mg/L. The influence of vintage over TPC indicates that the 2019 vintage results in a higher amount, 1037.53 mg/L, compared with 841.08 mg/L.
***Merlot* red wines** from two different climatic regions, Kavaja, Csa, and Mati, Cfa climatic zone, indicate higher TPC (471.35 mg/L) from the Kavaja region compared with Mati, 294.03 mg/L. The 2020 vintage results in higher TPC values, 307.01± 5.51 mg/L compared with 281.06 ± 0.04 mg/L. Two white grape cultivars, *Shesh i bardhë* and *Cerruje*, indigenous of Central and Northern Albania wine regions, show high interest in wine production. TPC values in *Shesh i bardhë* wine samples were much higher (682.46 mg/L) than in *Cerruje* white wines (118.88 mg/L).

3.3. Phenolic Acids

Hydroxybenzoic acids: Gallic acid, present in wine, is a hydrolysis product of the condensed and hydrolyzable tannins (gallate esters), not in grape berries (Waterhouse, Sacks & Jeffery, 2016). In the Kallmet red wines, hydroxybenzoic acids and flavanols constitute the leading group, reaching up to 87.8% of phenolic compounds. A difference between Mirdita (83.0%) and Lezha (91.4%) was observed referring to the total phenolic compounds. By splitting this group as hydroxybenzoic acids, it was found that they constitute 64% of Kallmeti wines, with no difference between the two regions. Gallic acid was found in up to 47.4% of the total phenolic compounds from both regions and vintage, comprising the most abundant phenolic compound. A significant difference was found between Lezha (270.82±0.82 mg/L) and Mirdita (204.95±4.96 mg/L) from the same vintage, 2019. Differences among vintage, in gallic acid amount were non-considerable. Ethyl gallate ester (31.33±10.33 mg/L) was the second phenolic among hydroxybenzoic acids with considerable differences among the two regions, Lezha (44.94±0.18 mg/L) versus Mirdita (18.42±0.27 mg/L).

Hydroxybenzoic acids and flavan-3-ols in *Shesh i zi* red wines constitute the leading phenolic group, reaching up to 95.6% of total phenolics. The hydroxybenzoic acids group constitutes the highest levels by 75.51% of the total phenolic, the highest value compared to other wines in this study. The difference in the amounts between the two vintages was observed with the 2019 vintage, 993.78±23.23 mg/L, and the 2020 vintage, 802.12±6.92 mg/L. Compared with other phenolic compounds, gallic acid had the highest levels (55.8%) of the total phenolics from both vintages. The mean value of 524.31±27.42 mg/L GA in *Shesh i zi* red wines, while the second phenolic compound from this group was the 3-O-galloyl quinic acid (144.73±14.79 mg/L) found in both vintages. These values were much higher than Kallmet (17.55 mg/L) and *Merlot* wines (22.51 mg/L).

According to terroir and vintage, hydroxybenzoic acids and flavanols constitute the most abundant phenolic group in *Merlot* wines, reaching up to 92.61% of the TPC value. A difference between Mati (97.59%) and Kavaja (86.05%) was observed in the wine regions. Even total flavonols indicate significant differences between the two areas. Gallic acid was found in the highest levels compared with other phenolic compounds

(167.80 mg/L) in wines from the Kavaja region, compared with wines from the Mati region, 120.43±1.13 mg/L. The second phenolic belonging to this group was found in ethyl gallate, with considerable differences when comparing two wine regions, Kavaja (54.12±0.18 mg/L) and Mati (33.02±0.34 mg/L).

Hydroxybenzoic acids (containing seven carbon atoms) and hydroxycinnamic acids (nine carbon atoms, phenylpropanoid derivatives) constitute two main groups of phenolic acids used for the wine quality and authenticity assessment. Hydroxybenzoic acids in *Shesh i bardhë* white wines were found in the lowest percentage (40.97%) compared with other analyzed wines in this study. Gallic acid was found in the highest levels compared with other phenolic compounds, 182 ± 1.99 mg/L, from both vintages. Overall, the *Cerruje* white wines show lower amounts of phenolic compounds than *Shesh i bardhë* white wines.

Hydroxycinnamic acids and derivatives: Hydroxycinnamic acids, stilbenoids, and hydroxybenzoic acids are classified as non-flavonoids. (E)-3-phenyl prop-2-enoic acid, known by the trivial name cinnamic acid, is the group's main structure. Tartaric acid esters of caffeic, coumaric, and ferulic acids are found in grape berries (Waterhouse, Sacks, & Jeffery, 2016). Results indicate that the phenolic acids were the second group in the studied wines. Kallmet red wines have phenolic acids (11.08%), with significant differences among regions, Mirdita (15.23%), and Lezha (7.93%). *Trans*-caftaric acid was the primary phenolic acid, 27.73±8.31 mg/L, in wines of the 2019 vintage, with differences among two regions, Lezha (19.82±0.41 mg/L) versus Mirdita (34.77±1.99 mg/L). The second phenolic acid was found *trans*-coutaric acid (5.72±1.97 mg/L), with the same pattern as *trans*-caftaric acid for two regions. Among tartaric acid esters of the phenolic acids, domination of *trans*- versus *cis*- isomers was observed in *trans*-caftaric acid (27.73±8.31 mg/L) versus *cis*-caftaric acid (0.91±0.24 mg/L). Non-esterified phenolic acids resulted in much lower levels than tartaric acid esters of phenolic acids, e.g., p-coumaric acid (1.84±0.80 mg/L) versus *trans*-coutaric acid 5.72±1.97 mg/L).

The same pattern belonged to *Shesh i zi* red wines with phenolic acids (4.1%) as the second group. *Trans*-caftaric acid (26.13±2.52 mg/L) was the main phenolic contributing by 67.22% to the total phenolic acid group, followed by second *trans*-coutaric acid 3.10±0.71 mg/L, and the third *trans*-fertaric acid 2.19±0.46 mg/L. Comparing Kallmet and *Shesh i zi* red wines, the phenolic acids group was in higher levels, 48.80±9.92 mg/L and 38.87±3.42 mg/L, respectively, in contrast with their contribution in the amount of all phenolic compounds.

Phenolic acids in *Merlot* wines from the Kavaja region (8.20%) were much higher than in the Mati region (1.48%). Trans-caftaric acid was the main flavonol in the *Merlot* wines from the Kavaja region (21.15±1.25 mg/L), while the primary phenolic acid was the glutathione-caffeoyl tartaric acid 1.48±0.05 mg/L). No pattern was observed among cis versus *trans* isomers in phenolic acid esters in the *Merlot* wines from the Mati region. The total amount of phenolic acids present in *Merlot* wines were found at a similar level with Sheshi zi red wines and in lower values when compared with Kallmet wines.

Phenolic acids constituted the second group of phenolic compounds found in white wines from *Shesh i bardhë* cv. (6.61%), followed by flavonols (3.45%) and stilbenoids (0.15%). An essential difference in total phenolic acids was observed among two regions in the study Kavaja (64.05±3.29 mg/L) and Tirana (44.84±0.89 mg/L). *Trans*-caftaric acid was the main compound with a mean value of 42.37±3.36 mg/L from the Kavaja region and 23.42 ± 0.28 mg/L in wines from the Tirana region. HPLC analysis of *Cerruje* white wines indicates that phenolic acids constitute the second group from the phenolic compounds, with 28.04%, much higher than the contribution of this group to other wines presented in this study.

Procyanidins and flavan-3-ol monomers

Tannins are flavonoids that comprise two different classes: hydrolyzable and condensed tannins. Hydrolyzable tannins comprise gallotannins (gallic acid derivatives) and ellagitannins (ellagic acid derivatives). Condensed tannins, called proanthocyanidins, are oligomers or polymers of flavan-3-ols, depending on their degree of polymerization. Their presence influences wine's taste, bitterness, astringency, and color (Merkytė, Longo, Windisch, Boselli, 2020).

Catechin was the second phenolic compound found in levels 38.92 ± 13.87 mg/L, a mean value for Kallmet red wines, and a substantial difference between the two regions Mirdita (27.74± 3.41 mg/L) and Lezha (50.10 ± 10.19 mg/L). The mean value of Procyanidins B3, B1, B2, and B4 in Kallmet wines resulted in 40.41±25.21 mg/L. Meanwhile, it was found that differences among regions were 2.5 times higher, respectively Mirdita (24.12 mg/L) and Lezha region (56.69 mg/L).

Together with GA in *Shesh i zi* red wines, the 3-O-galloyl quinic acid and procyanidin B3 contributed to the total phenolic acids as the second and third phenolic compounds. The mean value of Procyanidins B3, B1, B2, and B4 in revealed 152.07 ± 36.69 mg/L. Meanwhile, it was found that the differences between the 2019

and 2020 vintages were, respectively, 182.76 ± 1.68 mg/L and 121.37 mg/L. Catechin in *Shesh i zi* red wines was lower (13.42 ±8.02 mg/L) than Kallmet wines (38.92±13.87 mg/L).

The mean value of procyanidin dimers B3, B1, B2, and B4 in *Merlot* wines resulted in 55.94±25.21 mg/L. Meanwhile, it was found that there was no difference among regions referring to the total phenolics, while procyanidin profiles are very different. Procyanidin B2 (31.07± 0.10 mg/L) in the Kavaja region differs from wines from the Mati region with procyanidin B3 (24.32 ± 0.31 mg/L).

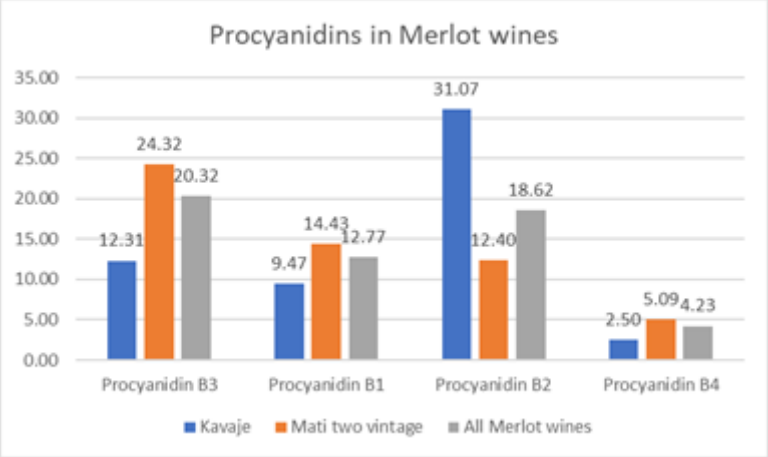

Figure 2. Procyanidins in *Merlot* wines (mg/L).

White wines from *Shesh i bardhë* cv. revealed procyanidin B3 as the second phenolic compound (142.90 ± 5.48 mg/L). The mean value of Procyanidins B3, B1, B2, and B4 was 246.25 ± 14.75 mg/L. The mean value of Procyanidins B3, B1, B2, and B4 in *Cerruje* white wines was much lower, 5.56 ± 0.06 mg/L. Procyaniding B2 was found at the highest level, 3.43 ± 0.03 mg/l, together with catechin 6.53 ± 0.21 mg/L and epicatechin 3.19±0.06 mg/L.

Table 4. Kallmeti red wines according to two climatic regions and two vintages(Mean±Stdev, mg/L).

Peak	**Compound**	**Mirdita**		**Lezha**		**Two vintages - two regions**
	Hydroxybenzoic acids and flavanols	**2019**	**2020**	**2019**	**2020**	
1	Gallic acid	175.14±4.78	204.95±4.96	245.28±1.60	270.82±0.82	224.05±39.35
2	3-O-galloyl quinic acid	18.52±2.89	23.59±0.97	13.77±1.00	14.32±0.09	17.55±4.38
3	Protocatechuic acid-O-hexoside	4.18±1.34	5.13±0.09	2.59±3.26	5.14±0.14	4.26±1.74
4	Gallocatechin	5.38±0.01	6.92±0.02	5.25±0.08	8.10±0.08	6.41±1.26
5	Protocatechuic acid	6.84±0.04	4.51±0.02	7.74±0.02	6.53±0.07	6.41±1.26
6	Epigallocatechin	1.09±0.00	1.40±0.01	1.04±0.01	1.66±0.01	1.30±0.27
7	Procyanidin B3	7.67±0.32	0.87±0.08	6.78±0.01	26.16±0.02	10.37±10.14
8	Procyanidin B1	4.51±0.11	5.24±0.28	2.72±0.00	16.51±0.14	7.24±5.80
9	Catechin	30.46±0.82	25.01±2.14	58.91±0.30	41.29±0.92	38.92±13.87
10	Procyanidin B2	12.44±0.07	11.91±0.04	19.19±0.05	28.00±0.29	17.89±6.96
11	Epicatechin	13.45±1.49	10.05±0.14	17.66±0.06	22.66±0.34	15.95±5.08
12	Procyanidin B4	2.52±0.02	3.08±0.03	6.38±0.02	7.64±0.05	4.91±2.31
13	Ethyl gallate	27.82±1.40	18.43±0.27	34.15±0.83	44.94±0.18	31.33±10.33
	Total Hydroxybenzoic acids and flavanols	**310.01±5.23**	**321.10±8.23**	**421.46±5.09**	**493.77±2.38**	**386.58±80.92**
	Phenolic acids					
14	2-S-glutathionyl-caffeoyltartaric acid	2.28±0.54	1.80±0.09	1.34±0.19	2.88±0.10	2.08±0.65
15	*cis*-Caftaric acid	1.00±0.45	0.75±0.23	1.02±0.08	0.86±0.18	0.91±0.24
16	*trans*-Caftaric acid	34.77±1.99	36.00±2.79	19.82±0.41	20.320.48	27.73±8.31
17	*cis*-Coutaric acid	2.04±0.16	1.54±0.02	2.54±0.35	1.71±0.11	1.96±0.43
18	*trans*-Coutaric acid	7.44±0.02	7.60±1.05	3.84±0.13	3.99±0.08	5.72±1.97
19	*cis*-Fertaric acid	3.27±0.02	2.77±0.00	2.31±0.02	2.12±0.00	2.62±0.48

20	*trans*-Caffeic acid	3.32±0.00	3.10±0.01	3.58±0.02	3.14±0.04	3.29±0.20
21	*trans*-Fertaric acid	2.69 ±0.00	2.45±0.00	3.15±0.00	2.38±0.01	2.67±0.32
22	p-Coumaric acid	1.48±0.01	1.46±0.00	1.28±0.00	3.13±0.02	1.84±0.80
	Total Phenolic acids	**58.30±2.78**	**57.48±3.95**	**38.88±0.21**	**40.54±0.94**	**48.80±9.92**
	Flavonols					
23	Quercetin-3-O-galactoside	1.09±0.01	0.83±0.03	0.21±0.00	1.62±0.07	0.94±0.54
24	Quercetin-3-O-glucoside	0.93±0.00	0.73±0.00	0.25±0.00	0.27±0.00	0.54±0.31
25	Quercetin-3-O-glucuronide	3.01±0.01	3.02±0.02	0.56±0.00	0.56±0.00	1.78±1.31
26	Isorhamnetin-3-O-glucoside	0.31±0.00	0.39±0.01	0.17±0.00	0.08±0.00	0.24±0.13
27	Quercetin	0.66±0.02	0.44±0.01	0.20±0.00	0.83±0.04	0.54±0.25
	Total Flavonols	**6.00±0.02**	**5.40±0.08**	**1.39±0.01**	**3.36±0.11**	**4.04±1.94**
	Stilbenoids					
28	*cis*-Piceid	0.31±0.00	0.43±0.00	0.31±0.01	0.76±0.01	0.46±0.20
29	*trans*-Piceid	0.45±0.00	0.48±0.00	0.28±0.00	0.78±0.01	0.50±0.19
30	*cis*-Resveratrol	0.01±0.00	0.01±0.00	0.01±0.00	0.00±0.00	0.01±0.00
31	*trans*-Resveratrol	0.09±0.00	0.12±0.00	0.08±0.00	0.05±0.00	0.09±0.03
	Total Stilbenoids	**0.87±0.01**	**1.04±0.00**	**0.68±0.01**	**1.59±0.02**	**1.04±0.36**
	Total Phenolics	**16.95±13.89**	**26.34±0.18**	**31.37±1.39**	**40.37±0.74**	**440.47±70.93**

In the red wines group, comparing the mean values of monomer flavan-3-ols, catechin, and epicatechin, indicate that the highest value reached *Kallmet* wines belonging to Lezha region 76.57±0.25 mg/L. This value was much lower in *Shesh i zi* wines, 22.81±11.76 mg/L. *Merlot* red wine from the Kavaja region reached the highest levels of 80.65±0.68 mg/L. Considerable differences were observed regarding region when comparing the same grape for the *Kallmet* and

Table 5. Shesh i zi red wines according to two vintages (Mean±Stdev, mg/L).

Peak	**Compound**	**KAVAJA**		**Two vintages**
	Hydroxybenzoic acids and flavanols	**2020**	**2021**	
1	Gallic acid	548.02±1.72	500.59±1.52	524.31±27.42
2	3-O-galloyl quinic acid	157.45±3.09	132.02±0.81	144.73±14.79
3	Protocatechuic acid-O-hexoside	35.46±44.73	2.89±0.08	19.18±31.94
4	Gallocatechin	9.91±3.23	5.36±0.06	7.63±3.22
5	Protocatechuic acid	12.30±0.03	2.38±0.02	7.34±5.73
6	Epigallocatechin	12.42±0.08	8.88±0.06	10.65±2.04
7	Procyanidin B3	136.30±1.57	97.12±0.08	116.71±22.63
8	Procyanidin B1	12.77±0.01	13.83±0.12	13.30±0.61
9	Catechin	16.32±11.73	10.52±4.68	13.42±8.02
10	Procyanidin B2	24.59±0.07	4.75±0.05	14.67±11.46
11	Epicatechin	6.49±0.02	6.54±0.10	6.51±0.07
12	Procyanidin B4	9.10±0.03	5.67±0.04	7.38±1.98
13	Ethyl gallate	12.66±0.07	11.63±0.04	12.14±0.60
	Total Hydroxybenzoic acids and flavanols	**993.78±23.23**	**802.18±6.92**	**897.98±111.50**
	Phenolic acids			
14	2-S-glutathionyl-caffeoyltartaric acid	4.80±0.69	3.69±0.13	4.24±0.76
15	*cis*-Caftaric acid	0.61±0.05	1.23±0.26	0.92±0.39
16	*trans*-Caftaric acid	28.28±0.59	23.99±0.56	26.13±2.52
17	*cis*-Coutaric acid	1.25±0.17	1.56±0.10	1.41±0.21
18	*trans*-Coutaric acid	3.71±0.12	2.48±0.05	3.10±0.71
19	*cis*-Fertaric acid	0.00±0.00	0.50±0.00	0.25±0.29
20	*trans*-Caffeic acid	0.09±0.00	0.50±0.01	0.30±0.24

21	*trans*-Fertaric acid	2.59±0.00	1.79±0.01	2.19±0.46
22	p-Coumaric acid	0.41±0.00	0.26±0.00	0.33±0.08
	Total Phenolic acids	**41.74±1.03**	**36.01±1.10**	**38.87±3.42**
	Flavonols			
23	Quercetin-3-O-galactoside	0.50±0.01	0.39±0.02	0.44±0.07
24	Quercetin-3-O-glucoside	0.35±0.00	0.40±0.00	0.38±0.03
25	quercetin-3-O-glucuronide	0.64±0.00	1.62±0.00	1.13±0.56
26	Isorhamnetin-3-O-glucoside	0.08±0.00	0.20±0.00	0.14±0.07
27	Quercetin	0.25±0.01	0.19±0.01	0.22±0.03
	Total Flavonols	**1.82±0.01**	**2.81±0.03**	**2.31±0.57**
	Stilbenoids			
28	*cis*-Piceid	0.00±0.00	0.00±0.00	0.00±0.00
29	*trans*-Piceid	0.03±0.00	0.04±0.00	0.04±0.01
30	*cis*-Resveratrol	0.01±0.00	0.00±0.00	0.01±0.01
31	*trans*-Resveratrol	0.14±0.00	0.04±0.00	**0.09±0.06**
	Total Stilbenoids	0.19±0.00	0.09±0.00	0.14±0.06
	Total phenolics	**1037.53±24.28**	**841.08±7.99**	**939.31±114.38**

Merlot cultivars in the respective regions were included in the study.

No variation was observed when analyzing the results of *Shesh i bardhë* white wines according to the vintage, but considerable compared to Tirana (83.92±2.07 mg/L) versus Kavaja region (40.75±0.95 mg/L). *Cerruje* white wines revealed low levels of flavan-3-ol monomers catechin and epicatechin. The monomer flavan-3-ols, catechin, and epicatechin ratio reached 64.92% of the total flavan-3-ols, including all forms of proanthocyanidins. Much higher than this value in *Shesh i zi* red wines but comparable with *Merlot* wines from the Kavaja region (54.08%). This ratio in the *Shesh i bardhë* white wines also revealed a low value (25.47%). The proanthocyanidin dimers in the total of the flavan-3-ols were relatively low in *Kallmet*i wines, with the highest value of 51.52% in wines from the Lezha region in the 2020 vintage. In contrast, in *Shesh I zi* wines, this indicator reached the maximum, with 80.38% from the 2019 vintage. *Shesh i bardhë* white wines had a high value, 78.29% from the 2020 vintage.

Different factors, such as climatic, geographical, or vintage, could determine the flavonoid biosynthesis, consequently, the proanthocyanidin levels in the grape berry (Jordao & Ricardo da Silva, 2019).

Comparison of *Kallmet* wines' results for flavan-3-ol monomers, catechin, epicatechin, epigallocatechin, and gallocatechin, with wines from international winegrapes, such as *Merlot*, Cabernet Sauvignon, Syral, Tempranillo, and Nero D'Avola, indicate that *Kallmet* wines presented similar values with Cabernet Sauvignon (Gutierrez, Lorenzo, & Espinosa, 2005). There was a considerable difference among flavan-3-ols (catechin epicatechin) when compared with Nero D'Avola: catechin (25 mg/L), epicatechin (32 mg/L) (La Torre, Saitta, & Vilasi, 2006). Compared with Syrah wine, lower values were found for both catechin (43 mg/L) and epicatechin (51 mg/L) (Gutierrez, Lorenzo, & Espinosa, 2005), higher catechin amounts compared with *Merlot* wines (27 mg/L), and similar values referring to epicatechin (19 mg/L) (Monagas, Suarez, Gomze-Cordoves, & Bartolome, 2005). Compared to the Tempranillo wine grape, catechin (27 mg/L), and epicatechin (54 mg/L), revealed the reverse catechin and epicatechin amounts in *Kallmet* red wines (Gutierrez, Lorenzo, & Espinosa, 2005).

Catechin (13.42 mg/L) and epicatechin (6.51 mg/L) in the *Shesh i zi* red wines were found in lower amounts referring to *Merlot* wines from Spain (Monagas, Suarez, Gomze-Cordoves, & Bartolome, 2005), Nero D'Avola wines, from Sicily (La Torre, Saitta, & Vilasi, 2006), and Cabernet Sauvignon, Syrah and Tempranillo wines from (Gutierrez, Lorenzo, & Espinosa, 2005).

Merlot wines analysis revealed that flavan-3-ol monomers, catechin (27.32 mg/L) and epicatechin (19.34 mg/L), high similarity with *Merlot* wines from Spain, catechin (27 mg/L), epicatechin (19 mg/L) (Monagas, Suarez, Gomze-Cordoves, & Bartolome, 2005).

The presence of flavan-3-ol monomers in *Shesh i bardhë* white wines shows that catechin (35.59±14.89 mg/L) and epicatechin (20.50±6.92 mg/L) were found in similar levels with *Merlot* wine (Monagas, Suarez, Gomze-Cordoves, & Bartolome, 2005), and Cabernet Sauvignon (Gutierrez, Lorenzo, & Espinosa, 2005), but the considerable difference with other wine grape cultivars like Nero D'Avola (La Torre, Saitta, & Vilasi, 2006), Syrah and Tempranillo (Gutierrez, Lorenzo, & Espinosa, 2005).

Flavan-3-ol monomers in *Cerruje* white wines, catechin (6.53±0.21 mg/L), and epicatechin (3.19±0.06 mg/L) were much lower than *Merlot*, Cabernet Sauvignon, NeroD'Avola, Tempranillo and Syrah. Compared with Greek wines (11.8–40 mg/L), the catechin amounts in Albanian red and white wines were comparable, except for the *Cerruje* white wines (Proestos et al., 2005). Finally, the flavan-3-ol monomers and oligomers contribution in the total phenolics according to the wine was *Kallmet* wines from Mirdita region (18.70%), *kallmet* wines from Lezha region (26.85%), *Shesh i zi* (20.07%), *Shesh i bardhë* (48.37%), *Cerruje* (12.85%) and *Merlot* (31.63%).

Flavonols

Flavonols are the most diverse group of non-polymeric flavonoids in grapes and wines as flavonol aglycones and glycosylated forms. 3-O-glucosides and 3-O-glucuronides are the main glycosides in wines. Flavonol concentration in wines depends on skin concentration, indicating that red wines have higher amounts than white wines (Castillo-Munoz, Gomez-Alonso, Garcia-Romero, Hermosin-Gutierrez, 2007). Flavonols are UV sunscreen compounds in berry skin (Waterhouse, Sacks & Jeffery, 2016).

Flavonol glycosides identified in the Albanian wines were, quercetin-3-O-galactoside, quercetin-3-O-glucoside, quercetin-3-O-glucuronide, and isorhamnetin-3-O-glucoside. The level of total glycosides falls in the range of published data for red wines (Jeffery, Parker, Smith, 2008; Waterhouse, Sacks & Jeffery, 2016). *Merlot* wines revealed the highest total glycoside flavonols, 23.93 ±0.05 mg/L. Among local cultivars, *Kallmet* red wine from the Mirdita region revealed the highest amount, 5.33 mg/L, while the minimum level reached the wines from the Lezha region (1.19 mg/L).

Table 6. Phenolic compounds in *Merlot* wines from two climatic regions (Mean±Stedev, mg/L).

Peak	Compound	KAVAJA	MATI		Two vintages - two regions
	Hydroxybenzoic acids and flavanols	**2021**	**2020**	**2021**	
1	Gallic acid	167.80±1.57	125.48±0.60	120.43±1.13	137.90±23.28
2	3-O-galloyl quinic acid	29.69±0.48	18.72±0.40	19.12±0.31	22.51±5.57
3	Protocatechuic acid-O-hexoside	1.92±0.02	6.40±0.35	6.27±0.05	4.86±2.28
4	Gallocatechin	11.18±0.04	14.06±0.11	9.89±0.03	11.71±1.91
5	Protocatechuic acid	2.96±0.01	5.82±0.03	6.58±0.02	5.12±1.71
6	Epigallocatechin	1.93±0.48	10.05±11.05	1.88±0.47	4.62±6.49
7	Procyanidin B3	12.31±0.05	24.21±0.59	24.43±0.11	20.32±6.21
8	Procyanidin B1	9.47±0.10	14.16±4.13	14.69±0.16	12.77±3.17
9	Catechin	52.72±1.25	15.02±3.36	14.21±0.34	27.32±19.75
10	Procyanidin B2	31.07±0.10	11.64±0.05	13.16±0.04	18.62±9.67
11	Epicatechin	27.92±0.57	17.30±0.64	12.79±1.24	19.34±6.98
12	Procyanidin B4	2.50±0.02	5.32±0.08	4.86±0.04	4.23±1.35
13	Ethyl gallate	54.12±0.18	33.02±0.34	26.00±0.12	37.71±13.09
	Total Hydroxybenzoic acids and flavanols	**405.59±0.89**	**301.18±5.55**	**274.30±0.02**	**327.03±62.09**
	Phenolic acids				
14	2-S-glutathionyl-caffeoyltartaric acid	1.76±0.04	1.48±0.05	1.35±0.03	1.53±0.19
15	*cis*-Caftaric acid	0.53±0.04	0.32±0.00	0.28±0.02	0.38±0.12
16	*trans*-Caftaric acid	21.15±1.25	0.71±0.00	0.64±0.04	7.50±10.59
17	*cis*-Coutaric acid	2.08±0.03	0.08±0.03	0.09±0.00	0.75±1.03
18	*trans*-Coutaric acid	5.88±0.39	0.30±0.08	0.33±0.02	2.17±2.88
19	*cis*-Fertaric acid	2.58±0.01	0.33±0.00	0.64±0.00	1.18±1.09
20	*trans*-Caffeic acid	1.23±0.01	0.23±0.00	0.15±0.00	0.54±0.54
21	*trans*-Fertaric acid	1.22±0.01	0.30±0.00	0.58±0.00	0.70±0.42
22	p-Coumaric acid	2.25±0.01	0.11±0.00	0.11±0.00	0.82±1.10
	Total Phenolic acids	**38.68±0.78**	**3.87±0.02**	**4.18±0.04**	**15.57±17.90**
	Flavonols				
23	Quercetin-3-O-galactoside	1.70±0.02	0.70±0.01	1.07±0.02	1.16±0.46

24	Quercetin-3-O-glucoside	6.86±0.01	0.22±0.00	0.33±0.00	2.47±3.40
25	Quercetin-3-O-glucuronide	13.48±0.03	0.50±0.00	0.43±0.00	4.80±6.72
26	Isorhamnetin-3-O-glucoside	1.89±0.03	0.06±0.00	0.05±0.00	0.67±0.94
27	Quercetin	2.27±0.03	0.35±0.01	0.54±0.01	1.05±0.95
	Total Flavonols	**26.20±0.13**	**1.83±0.02**	**2.43±0.03**	**10.15±12.43**
	Stilbenoids				
28	*cis*-Piceid	0.32±0.02	0.00±0.00	0.00±0.00	0.11±0.16
29	*trans*-Piceid	0.41±0.00	0.01±0.00	0.01±0.00	0.14±0.21
30	*cis*-Resveratrol	0.01±0.00	0.01±0.00	0.01±0.00	0.01±0.00
31	*trans*-Resveratrol	0.15±0.01	0.11±0.00	0.13±0.01	0.13±0.02
	Total Stilbenoids	**0.89±0.01**	**0.13±0.00**	**0.15±0.01**	**0.39±0.39**
	Total phenolics	**471.35±0.25**	**307.01±5.51**	**281.06±0.04**	**353.14±92.33**

The only aglycon identified in the analyzed wines was quercetin. Its levels were considered lower than the interval range in the red wines category (Waterhouse, Sacks & Jeffery, 2016). Meanwhile, Flavonol glycosides in white wines, *Shesh i bardhë* and *Cerruje*, show that the quercetin levels in white wines falling in the interval proposed, with one exception on *Shesh i bardhë* with the value found at 2.49 mg/L belonging to the 2020 vintage and Tirana region.

Flavonols in *Kallmet* wines were found in the levels 4.04±1.94 mg/L, with higher values in wines from the Mirdita region (5.70±0.35 mg/L), compared to wines from the Lezha region (2.37±1.14 mg/L).

Table 7. Phenolic compounds in Shesh i bardhë cv. and Cerruje white wines (Mean±Stdev, mg/L)

Peak	**Compound**	***Shesh i bardhë***				***Cerruje***
		Kavaja		**Tirana**	**Two vintages – two regions**	
	Hydroxybenzoic acids and flavanols	**2019**	**2020**	**2020**		**2020**
1	Gallic acid	221.44±5.36	215.82±14.20	182.97±1.99	206.74±19.80	50.77±0.26
2	3-O-galloyl quinic acid	20.41±0.84	16.54±2.58	16.82±0.15	17.92±2.28	1.35±0.01
3	Protocatechuic acid-O-hexoside	3.13±0.05	4.00±1.28	5.39±0.26	4.17±1.18	4.41±0.07
4	Gallocatechin	12.82±0.05	12.22±0.03	7.23±0.05	10.76±2.74	0.00±0.00
5	Protocatechuic acid	12.24±0.05	6.59±0.04	19.12±0.09	12.65±5.61	5.36±0.25
6	Epigallocatechin	20.96±0.11	18.44±0.02	12.83±0.02	17.41±3.72	0.00±0.00
7	Procyanidin B3	208.83±6.42	215.23±8.92	142.90±5.48	188.99±36.23	2.13±0.04
8	Procyanidin B1	16.39±0.86	21.85±0.51	17.44±0.16	18.56±2.63	0.00±0.00
9	Catechin	26.13±2.24	25.89±0.70	54.76±0.24	35.59±14.89	6.53±0.21
10	Procyanidin B2	24.48±0.09	13.19±0.08	38.24±0.18	25.31±11.22	3.43±0.03
11	Epicatechin	17.48±0.24	14.86±1.65	29.15±2.31	20.50±6.92	3.19±0.06
12	Procyanidin B4	5.99±0.06	7.36±0.07	26.84±0.09	13.40±10.43	0.00±0.00
13	Ethyl gallate	34.97±0.28	26.25±0.84	54.98±0.63	38.73±13.18	6.07±0.06
	Total HO-C_6H_4-COOH & flavanols	**625.27±16.16**	**598.23±6.52**	**608.68±9.87**	**610.73±15.13**	**83.23±0.86**
	Phenolic acids					
14	2-S-glutathionyl-caffeoyltartaric acid	1.97±0.10	2.03±0.48	3.67±0.24	2.56±0.90	0.03±0.01
15	*cis*-Caftaric acid	0.89±0.27	1.06±0.47	0.96±0.08	0.97±0.26	0.73±0.01
16	*trans*-Caftaric acid	43.37±3.36	43.03±2.47	23.42±0.28	36.61±10.38	20.93±0.62
17	*cis*-Coutaric acid	1.00±0.01	1.42±0.11	2.56±0.27	1.66±0.73	2.68±0.01
18	*trans*-Coutaric acid	5.98±0.82	6.84±0.02	7.43±0.03	6.75±0.75	4.55±0.01
19	*cis*-Fertaric acid	5.42±0.00	0.51±0.00	0.54±0.00	2.16±2.53	0.64±0.01
20	*trans*-Caffeic acid	3.15±0.01	3.39±0.00	2.03±0.00	2.85±0.65	0.51±0.01
21	*trans*-Fertaric acid	4.90±0.00	4.46±0.00	2.51±0.00	3.96±1.14	2.87±0.01
22	*p*-Coumaric acid	1.38±0.00	1.31±0.01	1.72±0.01	1.47±0.20	0.40±0.01

	Total Phenolic acids	**68.05±4.33**	**64.05±3.29**	**44.84±0.89**	**58.98±11.37**	**33.34±0.64**
	Flavonols					
23	Quercetin-3-O-galactoside	0.63±0.02	0.32±0.00	1.60±0.00	0.85±0.60	0.39±0.01
24	Quercetin-3-O-glucoside	0.91±0.01	1.51±0.00	6.21±0.01	2.88±2.60	0.34±0.01
25	Quercetin-3-O-glucuronide	3.27±0.02	3.45±0.01	10.89±0.01	5.87±3.89	1.21±0.01
26	Isorhamnetin-3-O-glucoside	0.42±0.01	0.87±0.00	2.20±0.01	1.16±0.83	0.13±0.00
27	Quercetin	0.32±0.01	0.36±0.01	2.49±0.02	1.06±1.11	0.23±0.01
	Total Flavonols	**5.55±0.08**	**6.51±0.02**	**23.40±0.05**	**11.82±8.98**	**2.31±0.05**
	Stilbenoids					
28	*cis*-Piceid	0.07±0.00	0.06±0.00	0.08±0.00	0.07±0.01	0.00±0.00
29	*trans*-Piceid	0.70±0.00	0.68±0.01	0.88±0.01	0.75±0.10	0.00±0.00
30	*cis*-Resveratrol	0.01±0.00	0.01±0.00	0.01±0.00	0.01±0.00	0.00±0.00
31	*trans*-Resveratrol	0.12±0.00	0.10±0.00	0.07±0.00	0.10±0.02	0.00±0.00
	Total Stilbenoids	0.90±0.00	0.86±0.01	1.04±0.01	0.93±0.08	0.00±0.00
	Total Phenolic compounds	**699.78±11.91**	**669.65±3.24**	**677.96±9.01**	**682.46±15.50**	**118.88±0.26**

The flavonols in *Shesh i zi* red wines were 2.31 ±0.57 mg/L, lower than *Kallmet* wines, 5.70±0.35 mg/L. The highest amount (2.81±0.03 mg/L) belonged to the 2020 vintage.

Flavonol profiles from *Merlot* wines in two climatic regions, Kavaja and Mati, indicate a considerable difference by supporting the role of flavonols as UV screeners. Total flavonols in *Merlot* wines from the Kavaja region were found in the levels 26.20±0.13 mg/L, compared with *Merlot* wines from the Mati region (2.43±0.03 mg/L).

Flavonol glycosides in *Shesh i bardhë* and *Cerruje* white wines are presented with the lowest percentage compared with other phenolic compound groups, hydroxybenzoic acid, flavan-3-ol, and phenolic acids, except stilbenoids. Total flavonols in *Shesh i bardhë* wines from the Kavaja region, 5.55±0.08 mg/L, were much higher than those from the Tirana region (23.40±0.05 mg/L).

Stilbenoids in red and white wines

Resveratrol is the main compound produced in grape berry skin. Both *cis/trans* isomers and their glucosides, *cis*-piceid and *trans*-piceid, were found in the wines. *Trans*-resveratrol biosynthesis in grape skin indicates that red wines have higher amounts than white wines.

Analysis of *Kallmet* red wine indicates the total stilbenoid content (1.04±0.36 mg/L) with a chemical profile of *trans*-resveratrol, *cis*-resveratrol, *trans*-piceid, and *cis*-piceid. A significant difference was observed between the two vintages, with higher levels in 2020, reaching above two folds. When compared among two climatic regions, Lezha and Mirdita, the wine samples from the Lezha region show higher amounts (1.59 mg/L) than (1.04±0.01 mg/L). *Trans*-piceid was the main stilbenoid, reaching up to 0.78 mg/L in the wine samples from the Lezha region.

Total stilbenoid amounts (0.14 ±0.06 mg/L) in *Shesh i zi* red wines were much lower than *Kallmet* wines (1.04±0.36 mg/L). *Trans*-resveratrol was the principal compound to the total. Compared with wines from North Macedonia, these compounds were lower, e.g., in Vranac wines, the *trans*-piceid (2.24±0.08 mg/L), and in *Merlot* wines, the trans-resveratrol (1.49±0.06 mg/L) (Kostandinovic et al., 2012).

The analysis of *Merlot* wines indicates that the total stilbenoids were lower, compared to *Kallmet* wines, according to climatic regions and vintages. Both wine cultivars present the same pattern when clustered in Lezha and Kavaja regions with climatic similarity and Mirdita with Mati, with very similar climatic conditions. The total stilbenoids (0.89±0.01 mg/L) from the Kavaja region were higher than wines from the Mati region (0.14±0.10 mg/L).

According to the literature, total stilbenoids in white wines may reach 0.5. mg/L (Romero-Perez, Lamuela-Raventos, Waterhouse, de la Torre-Boronat, 1996).

Total stilbenoids in *Shesh i bardhë* wines was 0.93 ±0.08 mg/L for both vintage and regions, much higher than the literature. Results on the stilbenoid profiles found that *trans*-piceid, the *trans*-resveratrol glucoside, was in the highest levels, 0.88±0.01 mg/L, in the 2020 vintage of the Tirana region. *Cerruje* white wines revealed no presence of resveratrol and other stilbenoids.

Principal component analysis (PCA) is a technique for reducing the dimensionality of such datasets, increasing interpretability, and minimizing information loss (Jolliffe & Cadima, 2016).

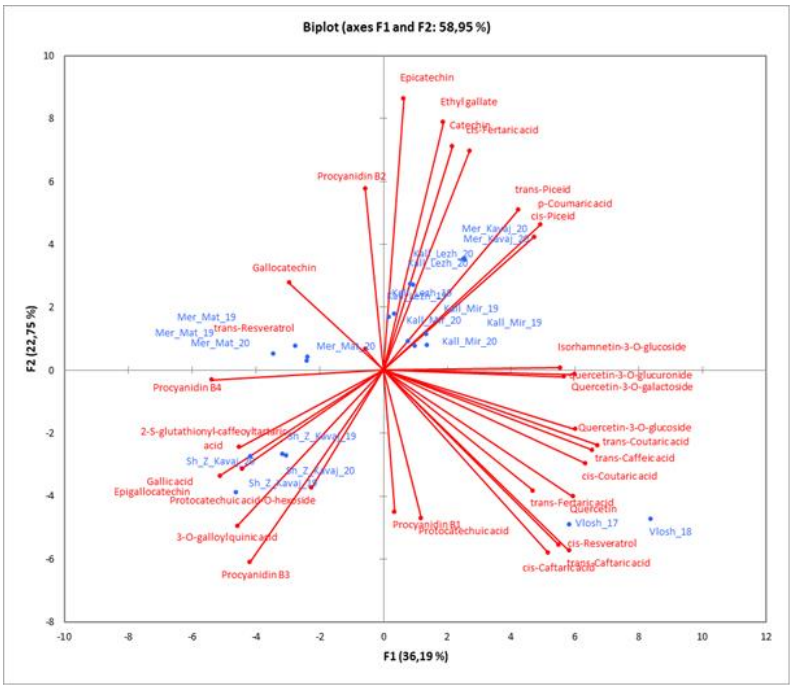

Figure 3. Biplot square Phenolic compounds (axes F1 and F2 in total 58.95%) and grape cultivars in red wines.

Flavonoids: Isorhamnetin-3-glucoside, quercetin-3-O-glucornide, and Qercetin-3-O-galactoside and Procyanidin B4 in the opposite contribute more in Principal Component 1 (PC1). In contrast, in the PC2 the main contributing attributions are nonflavonoids: epicatechin, ethyl gallate, and procyanidin B2, and on the opposite side, protocatechuic acid and procyanidin B1.

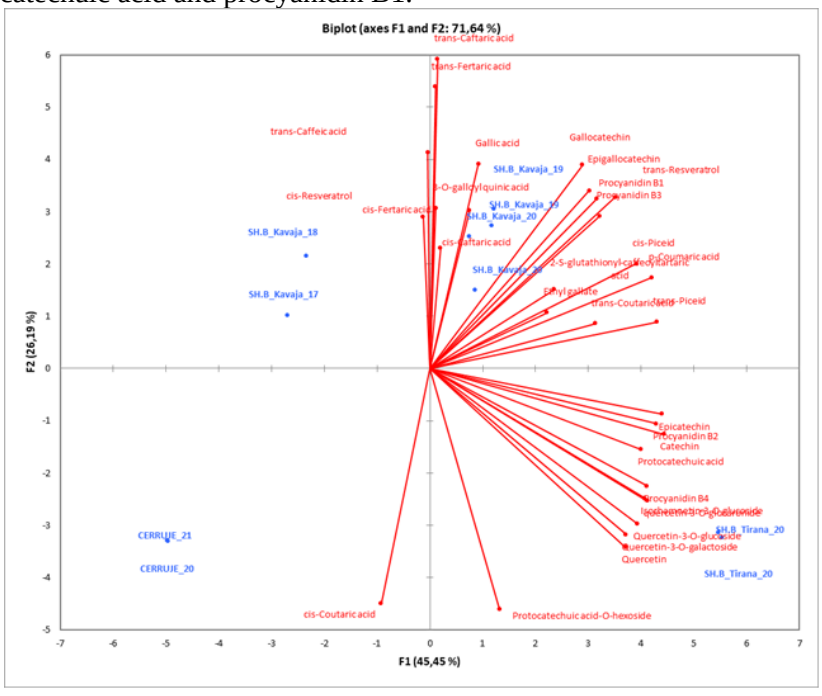

Figure 4. Biplot square (F1 and F2: 71.64%) for white wines according to vintage and terroir.

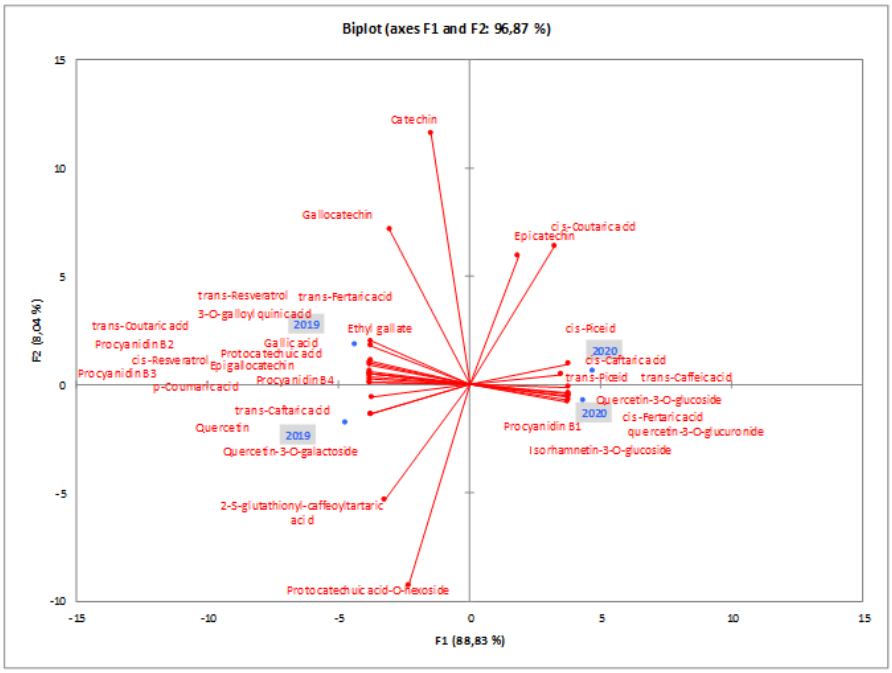

Figure 5. Biplot square (F1 and F2:96.87%) for wine phenolics in *Shesh i zi* red wines according to vintages.

PCA on *Shesh i zi* red wines belonging to two different vintages indicate that PC1 and PC2 contribute by 96.87%.

Cis and trans-piceid, Procyanidin B1, trans-caffeic acid, quercetin 3-O-glucoside, and quercetin-3-O-glucuronide serve as PC1 to the Sheshi izi wine samples of the 2019 vintage. Meanwhile, the PC1 to 2019 vintage may serve three other procyanidins B2, B3, and B4, together with gallic acid.

Kallmet red wines analyzed according to the terroir and vintage indicate good discrimination. PC1 and PC2: 8698%. Procyanidin B2 and B3 are good PC1 discrimination for *Kallmet* wine samples originating in the Lezha region and 2020 vintage. While the *Kallmet* wines that originate from Mirdita terroir, according to PC1 (53.33%), may be candidate markers cos and trans-resveratrol together with cis-fertaric acid and isorhametin-3-O-glucoside. Discrimination of *Kallmet* wines from the Lezha region to the 2019 vintage is discriminated according to PC2 (33.65%) according to trans-caffeic acid, cis-coutaric acid, trans-fertaric acid, protocatechuic acid, and catechin.

4. CONCLUSIONS

Wines phenolics in different wines produced by five winegrape cultivars, *Shesh i zi*, *Kallmet*, *Shesh i bardhë*, *Merlot*, and *Cerruje*, were analyzed. Vintage and climatic zones were two main factors together with grape cultivars are presented in this paper. Thirty-one flavonoids and non-flavonoids were identified in the wine samples. Liquid chromatography analysis indicates that winegrapes from the Central Albania region, *Shesh i zi*, and *Shesh i bardhë* are distinguished for their high levels of phenolic compounds. Meanwhile, *Kallmet* red wines are very special to their resveratrol content. Gallic acid was identified as the most abundant phenolic, reaching the maximum levels in *Shesh i zi* red wines. Flavan-3-ol monomers, catechin, and epicatechin were present in all wines, with higher levels in *Merlot* wines from the Kavaja region. *Shesh i bardhë* white wines were characterized by procyanidin dimers (B1, B2, B3, and B4) in the highest amounts. In the group of red wines, *Shesh i zi* wines were identified with the highest levels of anthocyanidins. PCA analyses produced good discrimination results among wine samples belonging to different grape cultivars and wine samples from the same cultivar but from different regions. The promotion of native cultivars not only contributes to genetic diversity and will help the wine economy to be competitive in the global market.

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Detection of Additives in Food Matrice by The Application of Molecularly Imprinted Polymers

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ABSTRACT

Food additives that improve food's flavor and fragrance are crucial to the food industry. Nevertheless, a lot of food additives are dangerous to people and misused in food for financial gain. Various techniques are employed to determine food additives whose World Health Organization-established daily intake limits are sensitive, selective, and accurate. Because of their high selectivity and sensitivity, molecularly imprinted polymers, or MIPs, have become a popular and straightforward technique for the quick and easy identification of food additives from food samples.

Keywords: *Food additives, molecular imprinted polymers*

INTRODUCTION

Food safety is directly related to nutritional quality and human health. Accurate evaluation of analytes, such as the freshness of raw materials and the nutritional value of processed foods, food additives, microbial toxins, and antibiotic residues, is crucial for food safety (Cengiz et al.,2022). Food additives that increase the flavor of food and enrich its aroma are very important for the food industry. However, many food additives are improperly used in food for profit and are harmful to humans (Boekel, 2006). That is why the food industry is constantly developing effective analytical methods and technologies to ensure food safety and food quality.
One of these innovations, molecular imprinting, is a technology that enables synthesis with highly selective sites on target molecules. Research in this technology has rapidly expanded in recent years due to its potential applications in various sectors including pharmaceutical, chemical, engineering, materials science, and biotech industries. In addition, one of the areas where this technique shows remarkable potential is food analysis (Huang et al. 2021; Villa et al., 2021).

2 MOLECULARY IMPRINTED POLYMERS

Molecular imprinting is a technology that enables expression with highly selective sites for target molecules. Molecularly imprinted polymers are a class of cross-linked polymers that can bind a particular target compound with high specificity. The polymers are prepared in the presence of the target molecule and the target molecule is used as the template. The specific binding site complementing the target analyte is produced after the template has been removed from solid polymers (Lok et al., 2009). Molecular imprinting technology was first proposed by Wulf and Sarhan in 1972. It spread through the efforts of Mosbach and his colleagues in the 1980s.
Molecularly imprinted polymers have several advantages over conventional immunosorbents, such as high selectivity and affinity, high stability, easy preparation (Piletsky et al., 2006). Compared to biological receptors, they are resistant to harsh chemical environments, temperature and pressure without loss of activity (Lavignac et al., 2004). Furthermore, molecularly imprinted polymers can be stored for years without losing their affinity for the target analyte (Lok et al., 2009). For the production of a molecularly imprinted polymer, five different components must be considered. These are the following: template, functional monomer, cross-linker, solvent, and initiator (Brüggemann, 2003).
In general, the choice of functional monomer is determined by the template and its functionality. The cross-linker contributes 90% of the polymerizable groups, determining the "polymer chemistry" of the molecularly imprinted polymer. The solvent is in charge of pore formation within the polymer. The template must be soluble in solvent for successful printing (this may require heating the mixture). However, the solvent should

not compete with the functional monomer for template interaction (BrüggemannLok et al., 2009)
There are three different approaches to prepare molecularly imprinted polymers: Covalent Approach, semicovalent approach and non-covalent approaches. **In the covalent approach**, prior to polymerization, involves the formation of reversible covalent bonds between the template and monomers. After that, the template is removed by separating the covalent bonds. Because of the high stability of the template and monomer, the covalent approach allows for the formation of homogeneous binding sites. However, this approach is restrictive as the cleavage of covalent bonds is quite difficult. **The semicovalent approach** relies on noncovalent interactions for rebinding even though the functional monomer and template are covalently bound. **The non-covalent approach** is based on the formation of non-covalent interactions (e.g. hydrogen bonding, electrostatic interaction, hydrophobic interaction, Van der Waals forces and dipole-dipole bonds) between the template molecule and functional monomers before polymerization. The simplicity of this approach and the availability of various monomers that can interact with nearly any kind of template make it the most popular method for creating molecularly imprinted polymers (Lok et al., 2009, Shao et al., 2022).

Use of Moleculary Imprinted Polymers in Food Additives

Controlling the production and delivery of food products is very difficult in the era of global markets. Creating quick, dependable, and affordable analytical tests to detect food contamination is essential in this situation. The creation of analytical analyses that are reliable, quick, and affordable has become necessary (Ayerdurai et al., 2022). With their high affinities for target analytes and selectivity for molecular recognition, molecularly imprinted polymers, or MIPs, meet this need. In terms of size, shape, and placement of the recognition site, MIPs faithfully replicate natural molecular recognition (Manesiotis et al., 2012). These characteristics make MIPs important for selective analyses of different food products (Table 1).

Table 1. Use of mloecular ımprinting polymers in food (Manesiotis et. al, 2012)

Family of food analytes	**For instance**
Additives	Sweeteners, dyes, flavors, preservatives
Components	Sugars, peptides, proteins, vitamins, fats
Contaminants	Bacteria, dioxins, toxins
Minerals, metals	Metal ions
Pesticides	Triazines, phenylureas, carbamates
Pharmaceuticals	Antibiotics, steroids

Food additives are compounds added to food in order to preserve its flavor and improve its taste or appearance, thus making it more attractive to the consumer (Manesiotis et al., 2012). Food additives used by the food industry including food preservatives, dyes, and artificial fragrances, can be present in food in significant amounts (Ayerdurai et al., 2022). Various methods are used for sensitive, selective and accurate detection of food additives (Shao et al., 2023). Food additives can be quantified with the help of dummy templates. Since in most cases these additives are well known and approved for use in foodstuffs and are added according to a recipe or regulation, the need for their determination in a food sample is rather limited; thus, the number of applications of MIPs to their analysis is also small (Manesiotis et al., 2012).

Table 2. A summary of the application of MIPs in the analysis additives in food

Analyte	**Template**	**Food Sample**	**Linear range**	**Ref.**
Tatrazine	Tatrazine	Saffron tea, ice cream samples	1.8-10,7µg/L	Zoughi et al. (2021)
Sunset yellow	Sunset yellow	Fruit drink (Fanta),orange flavoured candy, orange-flavored jelly powder,	4,5nM-9,1µM	Boyandi and Ghanbari (2021)

		cheese snack, and orange juice		
Tatrazine	Tatrazine	Soft drink	1.0-12,57µmol/L	Ruiz-C´ordova et al. (2021)
Butylated hydroxyanisole (BHA)	BHA	Chewing gum, mayonnaise, and potato chips	0.01–20 µg mL	Motia et al. (2021)
Vanillin, ethyl vanillin, maltol, ethyl maltol	3-hydroxy-2,6-bis(hyfroxymethyl)-4-pyrone	Beverage, orange juice nenergy beverage, coffe, tea, wine	2,07-2,71 ng/mL	Fu et al. (2019)
Methyl anthranilate	Methyl anthranilate	Wine	0,1-10 nM	Wei-Zhen et al. (2019)
Tertiary butylhydroquinone (TBHQ)	Tertiary butylhydroquinone (TBHQ)	Edible oil	0.5–60 µg/mL	Yue et al. (2019)
Amaranth	Amaranth	Soft drink	0.006–10 µM	Li et al.(2019)
Sudan dyes	1-(2-pyridinylazo)-2-naphthol	Egg	0.005-100 ng/mL	He et al. (2019)
Rhodamine B	Rhodamine B	Chilli powder	0,001-20,0 µg/mL	Zhai et al.(2017)
Fancy red	Fancy red	Chilli	0.010-0.250 mg/kg	Long et al. (2009)
BHA , BHT, PG	BHA, BHT, PG	Apple juice	1–0.01 mg/ml	Brüggeman et al. (2004)

3 CONCLUSION

In conclusion, MIP-based analytical techniques offer a comparatively straightforward analysis and have been effectively used to food additives with increased concentration and selectivity recently. It is anticipated that these techniques will soon have a wider range of application possibilities. The use of novel and biobased materials through a variety of polymerization techniques that facilitate more easily controlled adsorption and desorption of target analytes and active ingredients will be preferred by almost every industry. When planning the future of MIP synthesis, one important issue to take into account is the possible application of natural polymers and environmentally friendly reactants.

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A comparative study on Phytochemical evaluation of *Citrus auriantium* and *Citrus paradisi* juices)

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ABSTRACT

Citrus has been utilized globally to produce various value-added and nutritionally enhanced food products, including juices, wines, jams, canned citrus, and dried citrus. Citrus juices are consumed primarily due to their nutritional value and unique flavor. These juices are a significant source of bioactive components containing nutritional and health-promoting metabolites, including organic acids and phenolic compounds. Thanks to its chemical composition, it has a number of beneficial effects on health, including antioxidant, anti-inflammatory, antitumor, and antimicrobial activities. Naringin has various biological and pharmacological properties, such as protective effects against cardiovascular and neurodegenerative diseases, anti-carcinogenic, lipid-lowering, anti-apoptotic and anti-oxidant activities. To increase our knowledge on this subject, the characterization and quantification of *Citrus aurantium*, *Citrus paradisi*, Star ruby juices (which are the species of *Citrus paradisi*), were analyzed using HPLC-DAD-ESI-MS/MS. As a result of the analysis, it was found that 8 phenolic compounds were identified in all samples. However, *Citrus paradisi* and its species, Star ruby, detected 12 phenolic compounds. Naringin was determined as the dominant phenolic compound in *Citrus paradisi*, Star ruby, and *Citrus aurantium*. The amounts were determined to be 386.86±0.97 mg/L, 692.89±2.53 mg/L, and 602.97±1.62 mg/L, respectively. While neohesperidin (559.1±3.53 mg/L) was found in high amounts in *Citrus aurantium*, gallic acid, quercetin 3-O-glucoside(ısoquercitrin), didymin and poncirin compounds could not be detected. In addition, high amounts of quercetin-3-O-rutinoside (337.79±1.79 mg/L) and hesperidin (59.39±0.12 mg/L) were found in *Citrus Aurantium*. The dominant compound in Star ruby was narirutin (178.47±2.66 mg/L), followed by *Citrus paradisi* (122.22±0.56 mg/L). This study has shown that citrus juices may be a promising source of phenolic compounds that could support healthy dietary habits.

Keywords: *citrus juice, HPLC-DAD-ESI-MS/MS, phenolic compounds, naringin*

INTRODUCTION

Plant flavonoids comprise a diverse compound group that shares the common phenol moiety feature. They are typically plant metabolites resulting from the shikimate pathway and the phenylpropanoid metabolism, with a few notable exceptions (Harborne, 2013). Citrus fruit production has been global, with its biological and physiological significance contributing to producing a wide range of value-added and nutritionally enhanced food products, including juices, wines, jams, and canned and dried citrus. Citrus juices are consumed mainly because of their nutritional value and special flavor. They are significant sources of bioactive components with nutritional and health-promoting metabolites, such as organic acids and phenolic compounds. Citrus fruits and juices stand out among the most common phenolic rich dietary sources. It consist of volatile oils, limonoids, coumarins, alkaloids, sterols, and carotenoids in addition to vitamins (especially vitamin C), minerals and dietary fibers which make citrus a health-benefit-promoting fruit (El-Sayed et al., 2017). Flavonoids are secondary metabolites of plants that are members of the polyphenolic family. They are a remarkable and significant class of natural chemicals frequently found in fruits, vegetables, and some beverages Brodowska (2017). This is due to their ability to regulate critical cellular enzyme activities, as well as their antioxidative, anti-inflammatory, antimutagenic, and anticarcinogenic, cardio and covid-19 protective effect characteristics (Abotaleb et al., 2018; Cushnie et al., 2005; Qurtam et al., 2021; Chen et al., 2020; Mahmoud et al., 2019; Hou et al., 2019). They are one of the most unique classes of low-molecular-weight phenolic compounds found in higher plants.

Phenolic compounds contain important antioxidant and free radical scavenging compounds. The bioactivities of citrus juice have been linked to their phenolic composition, specifically flavonoid glycosides. Flavonoids

are mainly present in citrus fruits as their glycosyl derivatives. Aglycones (the forms lacking the sugar moieties) are less frequently present in juices due to their lipophilic nature and low solubility in water. Citrus flavonoids have pharmacological effects on inflammation, reactive oxygen species, blood lipids, and cholesterol levels (Barreca et al., 2017). Molecules such as luteolin and apigenin have been studied for their involvement in epigenetic treatment and cell gene expression through the modulation of HDAC1, HDAC2, and MMP-2 (Velmurugan et al., 2020).

Flavonoids can enhance the quality of fruit and juice in numerous ways, influencing the plant product's appearance, taste, and nutritional value. For instance, the presence of hesperidin in lemon and orange juices can cause sediment formation, resulting in undesirable cloudiness. Whereas the presence of naringin significantly influences the bitterness of grapefruit and bergamot juices (Mizrahi & Berk 1970; Guadagni et al., 1973).

Citrus paradisi, a species of the citrus family, is of great interest due to the presence of a number of essential components with antioxidant, cardiovascular, and antihypertensive properties (Fakhari et al., 2005). Naringin, present in considerable quantities in *Citrus paradisi*, has antiallergic, anticancer, antidiabetic, hepatoprotective, and respiratory effects. It also prevents cardiovascular diseases (Chtourou et al., 2015; Chen et al., 2016; Ahmed et al., 2019; Moghaddam et al., 2020). The effects of naringin on insulin resistance and diabetes have been documented by many *in vivo* and *in vitro* studies (Maity et al.,2017; Adeneye et al.,2008). In a study comparing the free radical scavenging activity of *Citrus paradisi* extract and naringin, it was stated that both showed antioxidant activity, and naringin had a higher radical scavenging effect compared to *Citrus paradisi* extract (Roghini and Vijayalakshmi 2018). According to the study based on the antioxidant, free radical scavenging and metal chelating properties of naringin, naringin increases cell durability by reducing the genotoxic effects of bleomycin (an antitumoral drug used in cancer treatment) (by reducing radiation-induced micronucleus formation and chromosomal fragmentation), thus making it a chemoprotective agent for use in clinical situations (Jagetia and Jagetia 2007). In another study investigating the antimicrobial activity of naringin against *P. aeruginosa* biofilm, it was found that naringin exhibited antimicrobial properties. Furthermore, when combined with antibiotics such as ciprofloxacin or tetracycline, naringin showed enhanced antimicrobial properties (Dey et al., 2020). Numerous preclinical reports indicate that naringin significantly impacts various applications dealing with bone diseases or stem cells for osteogenic differentiation, in addition to its many bioactive properties, including antioxidant activity (Sharma et al., 2021). Studies indicate that naringin has neuroprotective properties against several brain injuries by reducing oxidation- and inflammation-mediated changes. (Golechha et al., 2011; Cui et al., 2014; Han et al., 2017, Gaur et al., 2009).

In light of these information, the present study aimed to characterize the phenolic profile of *Citrus aurantium*, *Citrus paradisi*, and Star ruby juice (which are a species of *Citrus paradisi*). High-performance liquid chromatography combined with a diode array detection system (HPLC-DAD) and electrospray ionization mass spectrometry (HPLC-ESI-MS/MS) were employed to analyze the samples

MATERIAL-METHOD

Sample Preparation

Citrus aurantium fruits were harvested in Adana, Turkey. In addition, grapefruit (*Citrus paradisi*) was purchased from a local market in Adana. Meanwhile, Star Ruby was obtained from the Cukurova University Subtropical Research Center in September 2020. After the samples were cleaned and dried, the water was squeezed manually, followed by centrifugation and filtration through a 0.45 membrane filter. Analysis was conducted using HPLC-DAD-ESI-MS/MS.

Chemicals

Gallic acid, quercetin 3-o-glucoside (isoquercitrin), ferulic acid, feruloyl-galactaric acid, luteolin-7-o-rutinoside, quercetin-3-o-rutinoside, narirutin, naringin, hesperidin, neohesperidin,
didymin, poncirin, formic acid, acetonitrile and methanol were supplied from Sigma Aldrich
(Sigma-Aldrich, Steinheim, Germany) and Merck (Darmstadt, Germany).

LC-DAD-ESI-MS/MS analysis of phenolic compounds

An Agilent 1100 HPLC system (Agilent Technologies, Palo Alto CA-USA) operated by Windows NT based ChemStation software equipped with a diode array detector (DAD), auto sampler, degasser, binary pump. The column used was a Phenomenex reversed-phase C-18 column: 4.6mm×250mm, 5 µm) (Torrance, CA, USA). The mobile phase consisted of solvents: Solvent A, water/formic acid (99:1; v/v), and Solvent B, acetonitrile/solvent A (60:40; v/v). The following conditions were used to elute phenolic compounds: The flow rate was set at 0.5 ml/min, and the temperature was set to 25°C. All peaks' ultraviolet-vis spectra (ranging from 200 to 600 nm) were recorded. The phenolics' retention times and UV spectra were compared to valid standards and then confirmed using an Agilent 6430 LC-MS/MS spectrometer equipped with an electrospray ionization source. The following optimum parameters were used in negative ion mode: Electrospray ionization mass spectrometry detection at 400°C capillary temperature, 12 L/min N2 drying gas, 45 psi nebulizer pressure. Quantification was performed by external calibration with valid standards. Phenolic compounds were eluted according to the method described by Kelebek et al. (2020). The standard curves were acquired utilizing the commercial standards at concentrations that normally exist in extracts (nearly 1–100 mg/L) and getting regression coefficients (r^2) above 0.995 in all cases. In the absence of reference compounds, the calibration of structurally related substances was used, considering the molecular weight correction factor.

Statistical analysis

Data was analyzed using SPSS 20.0 (SPSS Inc., Chicago, IL). Comparison between groups was assessed by one-way analysis of variance (One-way ANOVA) followed by the Duncan's Multiple Comparisons test.

RESULTS

By analyzing the phenolic compounds in the samples (Table 1), this study identified naringin as the dominant compound in *Citrus paradisi*, star ruby, and *Citrus aurantium* juices (Figure 1). The present amounts were 386.86±0.97 mg/L, 692.89±2.53 mg/L, and 602.97±1.62 mg/L, respectively. Wu et al (2007) reported a significantly lower amount of naringin (44.64 mg/L) in grapefruit juice when compared to our results. It is thought that this difference may be due to the different climate conditions and soil in which the samples were grown. In parallel with our study, the phenolic composition of the juices of different grapefruit species was investigated and the most dominant compound was found to be naringin (464±1.62 mg/L) (Kelebek, 2010). In the study in which the phytochemical properties of different types of grapefruit juices were examined, the most dominant compound was found to be naringin, similar to our study, and its amount in Star Ruby was found to be 666± 22.63 mg/L (Rio et al., 2022). Also, the highest concentration of naringin is found in commercial grapefruit (*Citrus paradisi*) juice (435 mg/L), which is much more concentrated than the hand-squeezed juice equivalent (230 mg/L) (Gattuso et al., 2007).

While naringin (602.97±1.62 mg/L), neohesperidin (559.1±3.53 mg/L) were found in high amounts in *citrus aurantium*, gallic acid, quercetin 3-O-glucoside(ısoquercitrin), didymin and poncirin compounds were not be detected. In addition, *citrus aurantium* contained high amounts of quercetin-3-O-rutinoside (337.79±1.79 mg/L) and hesperidin (59.39±0.12 mg/L). Generally, neohesperidin, hesperidin, and naringin are the main compounds found in *citrus aurantium* (Hamdam et al., 2014). In Kawaii et al. (1999), naringin and neohesperidin had concentrations of 19.7 mg/L and 8.7 mg/L respectively in *C. aurantium* juice, but these values are significantly lower than those obtained in our study.

Table1. Retention times (Rt), mass spectral data for analyses of phenolic compounds in *citrus paradisi*, star ruby and *citrus aurantium* fruits juice in using LC-DAD-ESI-MS^n detection **(mg/L)**

RT (min)	Compound name	$[M-H]^-$ (m/z)	Fragment ions (m/z)	*Citrus paradisi* juice	Star ruby juice	*Citrus aurantium* juice
14.89	Gallic acid	169	137-125	0.24±0.04[a]	0.35±0.00[b]	nd

22.90	Quercetin 3-O-glucoside (Isoquercitrin)	463	301-268-179-151	68.88±1.77^{a}	90.26±1.26^{b}	nd
27.74	Ferulic acid	193	178-149-134	5.93±0.33^{c}	4.02±0.10^{b}	2.96±0.32^{a}
30.89	Feruloyl-galactaric acid	385	209-191	1.63±0.08^{a}	2.57±0.11^{b}	1.47±0.12^{a}
39.24	Luteolin-7-O-rutinoside	593	285	70.93±0.20^{c}	44.96±0.67^{a}	51.2±0.8^{b}
47.60	Quercetin-3-O-rutinoside	609	301-179-151	4.56±0.08^{a}	8.46±0.10^{b}	337.79±1.79^{c}
50.27	Narirutin	579	271	122.22±0.56^{b}	178.47±2.66^{c}	13.29±1.73^{a}
51.95	Naringin	579		386.86±0.97^{a}	692.89±2.53^{c}	602.97±1.62^{b}
53.48	Hesperidin	609	301-286-177-151	12.17±1.55^{a}	19.54±1.62^{b}	59.39±0.12^{c}
54.75	Neohesperidin	609	343-301	20.33±0.30^{a}	19.03±0.99^{a}	559.1±3.53^{b}
60.11	Didymin	593	285-245	2.05±0.09^{a}	5.14±0.34^{b}	nd
61.20	Poncirin	593	308-285	21.85±0.54^{a}	44.51±1.15^{b}	nd
	Total:			**717.65**	**1110.2**	**1628.17**

$^{a-b}$ Different exponential letters in the same line indicate a significant difference between the examples. (P<0.05)
nd: not detected

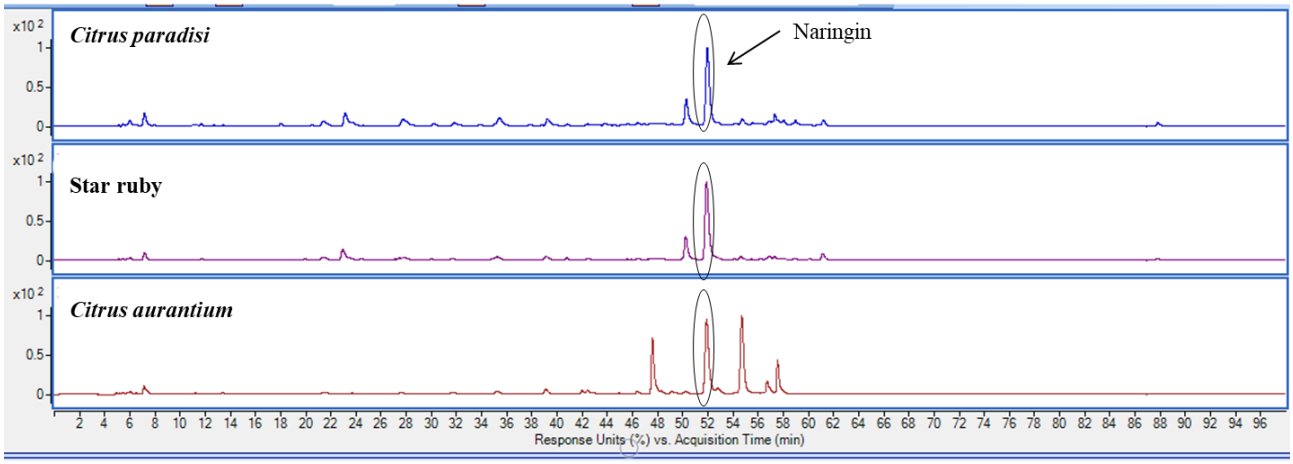

Figure 1. HPLC chromatograms of phenolic compounds in *citrus paradisi*, star ruby and *citrus aurantium* fruits juice

CONCLUSION

In the general evaluation of the results, a significant difference between the samples was observed. Naringin, which has many bioactive properties, was dominant all samples. It is thanks to these properties of naringin that we have a remarkable data on its purification and encapsulation for our future studies. This study has revealed that the composition of phenolic compounds in citrus juice samples is significant and may be used as a food supplement or ingredient due to the important antioxidant properties contributing to human health. However, additional *in vitro* investigations are required to assess the correlation between the chemical composition of citrus juice and its mechanisms of action in the treatment of various diseases.

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Development of experimental equipment for vegetables and fruits

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ABSTRACT

Drying of porous materials is a crucial and versatile process employed across various industries, including the food, paper, and pharmaceutical sectors. This multifaceted procedure hinges on the harmonious interplay of heat and mass transfer, primarily executed by directing streams of hot air onto a colder material, typically a product with high moisture content. The fundamental objective is to reduce the moisture content within the material while simultaneously elevating its temperature. These intertwined actions hold significant importance, ensuring the longevity and preservation of a wide range of products, most notably in the context of food preservation. In the food industry, the drying process is an indispensable method for extending the shelf life of perishable products. By expelling excess moisture from fruits, vegetables, meats, and grains, the proliferation of microorganisms and enzymatic activity is effectively curtailed. As a result, the risk of spoilage and bacterial growth diminishes, and the product becomes less susceptible to deterioration. This preservation technique is pivotal for producing a variety of food items, from dried fruits and jerky to pasta and cereal. Similarly, the paper and pharmaceutical industries rely on drying processes to achieve specific quality standards. In paper manufacturing, drying ensures the removal of water from the pulp, allowing for proper bonding of fibers and the creation of a coherent and printable product. In the pharmaceutical sector, controlled drying is instrumental in producing drugs, enabling precise formulation and preservation of active ingredients. Consequently, the drying of porous materials serves as a linchpin in multiple industries, enabling the production of durable and high-quality products while preventing spoilage and maintaining integrity.

Keywords: Drying, Porous Materials, Heat and Mass Transfer, Preservation

Energy efficient technology for drying food

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ABSTRACT

In contemporary times, the escalating demand for energy across diverse applications, such as the drying of textiles, food, paper, ceramics, and wood, underscores the growing significance of the drying process. This surge in energy consumption is prompting a reevaluation of the methods and technologies employed to achieve efficient drying, as it directly impacts both economic considerations and environmental sustainability. To put the energy implications into perspective, various sectors have established norms for acceptable energy consumption in drying processes. In the timber industry, for instance, energy consumption benchmarks have been set, and similar standards are in place for agricultural products, the paper industry, ceramics, building materials, textiles, and chemicals. The need for stringent norms arises from the fact that energy consumption in the drying process is inherently substantial, making it a focal point for cost control and ecological responsibility. This elevated energy usage has profound implications for the manufacturing sector, where the drying phase can account for a substantial portion of total production expenses. In fact, it's not uncommon for drying costs to encompass a significant share, ranging from 65% to 75%, of the overall production expenditure. Consequently, this places an urgent imperative on industries to innovate and adopt more energy-efficient drying techniques, not only to optimize operational costs but also to align with broader sustainability goals. In this context, research and development efforts are directed toward the development of cutting-edge technologies that reduce energy consumption and minimize the environmental footprint associated with drying processes.

Keywords: Energy Consumption, Drying Process, Energy Efficiency, Manufacturing Sector, Sustainability Goals

Method of storing agricultural products

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ABSTRACT

Various food preservation methods play a pivotal role in prolonging the shelf life of food products during storage. These techniques are designed to thwart microbiological changes while upholding the quality and safety of the items. The efficacy of these preservation methods is intricately linked to the maintenance of impeccable hygiene throughout the production process, as this is essential for diminishing the microbial load and preventing microbial proliferation. Often, the most commonly employed method for achieving this is heat treatment. However, it's worth noting that such high-temperature processing can bring about significant alterations in both the sensory attributes (texture, taste, and color) and the nutritional properties of the food, leading to a potential loss of essential vitamins. Recognizing the drawbacks associated with high-temperature processing, the food industry has been actively developing non-thermal preservation processes, often referred to as "soft technologies." These innovative techniques represent a milder approach, characterized by their less aggressive nature. One of their key advantages lies in their ability to yield food products that closely resemble fresh items, aligning perfectly with the evolving demands of today's market. Notably, these soft technologies ensure that the products maintain their safety guarantees, even as they deliver on the desire for food items that are not overly processed and retain their natural, fresh-like characteristics. This nuanced approach to food preservation demonstrates the industry's commitment to satisfying consumer preferences while prioritizing food safety.

Storing vegetables and fruits using ultraviolet light

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ABSTRACT

When vegetables are subjected to UV-C radiation, not only does this process effectively reduce the initial microbial load on their surfaces, but it also triggers an intriguing phenomenon known as the hormetic effect. This phenomenon is characterized by the stimulation of biological systems at low doses of a substance, which can subsequently lead to inhibition at higher doses. In the context of UV-C radiation, the hormetic effect carries significant implications for food safety and quality. One remarkable aspect of the hormetic effect in UV-C treatment of vegetables is its potential to enhance the produce's resistance to certain microorganisms, particularly molds and yeasts. This enhanced resistance can be attributed to the stimulation of specific enzymatic pathways. For example, exposure to UV-C radiation can stimulate the production of an enzyme known as phenylalanine ammonia lyase. This enzyme plays a pivotal role in the synthesis of phenolic compounds within the plant tissues. These phenolic compounds are known to be toxic to a wide range of microorganisms, including molds and yeasts. As a result, the induction of phenolic compound production through UV-C exposure can serve as a natural defense mechanism for the vegetables. These compounds act as protective agents, inhibiting the growth and proliferation of potentially harmful microorganisms that might cause spoilage or lead to foodborne illnesses. In summary, the hormetic effect induced by UV-C radiation not only reduces the surface microbial load on vegetables but also enhances their innate resistance to molds and yeasts through the stimulation of phenolic compound production. This dual benefit makes UV-C treatment a promising technology for improving the safety and shelf life of fresh produce.

Keywords: UV-C Radiation, Hormetic Effect, Vegetable Safety, Phenolic Compound Production

Fireweed (C*hamerion angustifolium* (L.) holub) and its biologically active organic compounds

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ABSTRACT

Fireweed (*Chamerion angustifolium* (L.) *Holub* or *Epilobium angustifolium* L.) is one of the most popular species of the genus Epilobium. Despite its widespread use in traditional medicine for its inflammatory, antimicrobial or antioxidant properties, most of the scientific work has been devoted to the analysis of its non-volatile organic compounds and their bioactivities. Research on the volatile organic compounds of this plant is still very scarce. This was the main impetus for the study, which aimed to assess the yield and chemical composition of essential oils extracted from fireweed flowers and leaves collected in different Lithuanian habitats, as well as the relationship between these parameters and the soil composition of the site, in addition to determine the antioxidant capacity of fireweed leaves aqueous extracts.

The essential oils of the flowers and leaves of the *Ch. angustifolium* (L.) *Holub* were extracted by hydro-distillation and analyzed by gas chromatography-mass spectrometry (GC-MS). Soil elemental analyses were done by Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) method. Total phenolic content (TPC) and antioxidant activity (AA) of aqueous extracts were determined.

Despite the high biomass content of narrow-leaved fireweed, the essential oil yield was negligible and depended mainly on the part of the plant: up to 0.05 % (v/w) for flowers and up to 0.8 % (v/w) for leaves. Essential oil compositions of both flowers and leaves are dominated by higher molecular weight compounds, long chain hydrocarbons, their esters, and alcohols. The most characteristic compounds in the essential oils of fireweed flowers and leaves were hexadecanoic acid, tetradecanoic acid and linalool. No correlation was found between the composition of the soil in the plant's natural habitat and its VOCs or TPCs.

Keywords: Antioxidant activity, essential oils, *Chamerion angustifolium* (L.) Holub, soil elements.

Characterization of biodegradable films prepared from chemically modified pearl millet starches

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ABSTRACT

Starch-based films possess potential application in food packaging due to their biodegradability, lower cost, stretchability and improved transparency. In the present study, novel edible films were developed from native and chemically modified pearl millet starches using solvent casting method. Three chemical modifications were employed namely: cross-linking, octenyl succinylation, and acid-thinning at a concentration of 3g/100 of starch. Glycerol (30% w/w) was used as a plasticizer. The microstructure, thickness, moisture content, water solubility, tensile strength, elongation-at-break, water vapor permeability (WVP), thermal transition temperatures (To and Tp), l*a*b color parameters and transparency (%T) of the prepared films were determined. Scanning electron microscopy (SEM) images showed that cross-linked (CLF), octenyl succinylated (OSF), and acid-thinned (ATF) starch films had homogeneous surfaces without pores or cracks when compared with native starch film (NF). Interestingly, films containing modified starches resulted in significantly improved moisture proof property in comparison to NF. With respect to WVP, ATF exhibited the least value (6.75 x 10-11 g.m-1.h-1.Pa-1) followed by CLF, OSF and NF. Also, CLF and OSF showed significantly reduced water solubility as compared to NF. According to Differential Scanning Calorimetry (DSC) thermograms, the onset temperature (To) of ATF was significantly lower in comparison to other films. Tensile test revealed that chemical modifications improved the mechanical strength of NF with CLF showing the greatest increment. The light transmittance of ATF was the highest among all films suggesting its application in packaging of food products with clear vision. Based on these results, it is concluded that, edible films prepared from aforementioned chemically modified pearl millet starches with attractive physical properties and eco-friendly nature could be efficiently utilized for food packaging purposes.

Keywords: acid-thinning, cross-linking, edible films, octenyl succinylation, pearl millet starch

Burden of disease estimation based on *Escherichia coli* quantification from ready-to-eat meals of institutional canteens

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ABSTRACT

Shiga toxin-producing *Escherichia coli* (STEC), enterotoxigenic *E. coli* (ETEC), and enteropathogenic *E. coli* (EPEC) are associated with the onset of gastroenteritis with different severities. Additionally, STEC has been associated with other sequelae, such as hemolytic uremic syndrome and end stage renal disease. This study's aim was to estimate the annual foodborne burden of disease associated with STEC, ETEC, and EPEC infection based on *E.coli* quantification results obtained in the routine analysis of ready-to-eat meals served in institutional canteens during a 2-year period.To attain that purpose, quantitative microbial risk assessment was selected and a stochastic model was used to estimate the expected number of cases per health outcome and Disability Adjusted Life Years (DALYs). In addition, a sensitivity analysis was also performed using the Sobol method. Considering the daily ingestion of a whole meal, the estimated burden for STEC infection was of 4.2 x 10-3 DALYs/person/year, while for ETEC infection a 2.82 x 10-4 DALYs/person/year was estimated, and for EPEC infection a 7.91 x 10-6 DALYs/person/year was calculated. The sensitivity analysis revealed that the factors that most contributed to the overall output variability were the pathotype's prevalence in the STEC model, the number of people exposed to the hazard in the ETEC model, and *E. coli* concentrations in the EPEC model. By combining quantitative microbial risk assessment and health metrics estimates, this study draws attention to an innovative approach that contributes to a better understanding of foodborne *E.coli* impact on consumers' health.

Keywords: Disability Adjusted Life Years, *Escherichia coli*, Quantitative Microbial Risk Assessment, Ready-to-eat foods.

Food safety and hygiene knowledge, attitudes, and practices assessment of industrial food handlers

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ABSTRACT

This study aimed to assess the food safety and hygiene knowledge, attitudes, and practices of food handlers from industrial units producing deep-frozen and ready-to-eat foods. To attain that aim, 95 food handlers from 3 industrial units located in Lisbon, Portugal were interviewed for sociodemographic data collection and food safety and hygiene knowledge, attitudes and practices assessment. Most food handlers were women (83%), aged between 40 and 59 years old (56%) with basic or lower education (61%) levels. Most food handlers had been working in the food industry for more than 5 years (73%) and had attended more than 5 training courses (71%) in food safety and hygiene. Considering the results of the knowledge assessment, a good compliance level (80%) was obtained, although particular questions presented poor results, such as those related to the effect of freezing in microorganisms' survival in foods, and to fruits and vegetables good handling practices. In the attitudes assessment, a high compliance level (95%) was obtained, revealing participants' positive attitudes regarding the importance of training in food safety and hygiene. In the self-reported practices assessment, a good compliance level (>80%) was also obtained. Overall, this study revealed a good level of knowledge, attitudes and practices in food safety and hygiene, although some specific aspects could be improved. To this end, training intervention strategies should be adapted, as a way of promoting and improving food safety awareness and culture among food handlers.

Keywords: Food hygiene, food safety, food handlers, food industry.

Comparative analysis of citric acid and hydroxypropylated modified sorghum and corn instant starches prepared via alcoholic alkaline treatment (aat) and extrusion (ex) technique

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ABSTRACT

The present study investigated the chemical and physical modification of sorghum and corn starches followed by comparative analysis in terms of their functional, morphological, thermal and rheological properties so as to suggest sorghum starch as a substitute of corn starch in forthcoming era. This study concluded that chemical modified instant starches develop from alcoholic alkaline treatment and extrusion technology process has quite different functional, morphological, thermal and rheological characteristics. The AAT treated sorghum and corn citrates possessed higher and rapid instant viscosity as compared to those prepared by extrusion technique. Comparatively, hydroxypropylated instant starches showed lacking in viscosifying properties either prepared via AAT or extrusion. The AAT treated instant starches showed relatively better functional properties as compared to extrudates either they are modified through conventional (hydroxypropylated) and non-conventional (citric acid) modification methods. Extrusion is relatively harsh treatment compared to AAT. Extruded instant starches showed intense fragmented granular structure due to high notch of shear. However, AAT instant starch does not involve intense fragmentation of granules due to less shear. Citric acid modification and hydroxypropylation prior to physical modification affects the gelatinization and retrogradation pattern of the starch. The hydroxypropylated starch extrudates exhibited higher swelling power along with lower rate of retrogradation as compared to citric acid modified starch extrudates. The FTIR and XRD of instant starches were performed to analyze the crystalline to amorphous ratio, the type of crystallinity and presence of V-type crystallinity. Therefore, it is obvious conclusion that the properties of the chemical modified starch could be tailored depending on the method using for preparation of chemical modified instant starches. However, there is a need to optimize these treatments when starches were obtained from different botanical sources.

Keywords: Alcoholic alkaline treatment, Extrusion, Instant starch, Sorghum.

The use of protein hydrolysates in the enrichment of confectionery products

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ABSTRACT

Both children and adults frequently consume confectionery products despite their relatively low nutritional values. Soft confectionery products contain sweeteners (sucrose and glucose syrup), gelling agents, coloring agents, flavor, and acids. The nutritional value of confectionery products can be improved by the addition of protein hydrolysates. This study aimed to prepare protein hydrolysates prepared via enzymatic hydrolysis of plant proteins and their utilization in confectionery products, and finally to investigate the bioactivity of the hydrolysates after simulated digestion. Sunflower and hazelnut cold-press cakes were used as the protein sources. Protein isolation was conducted by the alkaline extraction-isoelectric precipitation method. Protein Hydrolysates were obtained using trypsin and bromelain. Bioactivity tests (DPP-IV inhibitory activity and antioxidant activity tests) were administered to confectionery products bearing bioactive hydrolysates before and after in vitro simulated digestion. Dipeptidyl Peptidase-IV inhibitory activities of the digested confections ranged between 40.6 and 42.8%. The anti-diabetic activities of hydrolysate bearing confectionery increased after digestion. The anti-diabetic potential of peptides in the hydrolysates were affected by further degradation by simulated digestion. The total phenolic content of samples varied between 3.7±0.1 and 81.5±3.9 Gallic acid Equivalent mg/ 100 g before in vitro digestion. Antioxidant activities of confectionery samples were found in the range of 9.4±0.6 and 137.9±8.2 Trolox Equivalent mg/100 g and between 5.3±0.6 and 100.6±12.9 TE mg/100 g, respectively, based on CUPRAC and DPPH assays. Antioxidant capacities decreased for each confectionery sample after in vitro digestion. The total phenolic content and antioxidant capacity of confectionery products bearing sunflower seed protein hydrolysates were higher than hazelnut counterparts since sunflower seeds are rich in phenolic components.

Microwave-Assisted drying of mango peels: drying kinetics and optimization of process conditions using mathematical models and response surface methodology

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ABSTRACT

The effects of microwave drying (MWD) on the drying kinetics, phytochemical characteristics, and antioxidant activity of control and steam blanched mango peels were investigated for the first time through this work. Peels of uniform thickness (~1.5 mm) were used for drying experiments using 3 microwave power levels (360 W, 540 W and 720 W) for 7-9 minutes. The experimental data were best fitted using mathematical models (Page, Henderson and Pabis, Peleg and Lewis). The Page and Peleg models (R2 = 0.99) provided the best fit to the mango peel drying curve. To produce dried mango peel powder and its valorization, the trial runs for process parameters (microwave power and time) were performed using CCRD (Central Composite Rotatable Design). The responses measured were moisture content, drying rate, color, TPC, TFC, DPPH and FRAP. The results of the experiment were accurately predicted by a quadratic model with significant R2 values, according to ANOVA. The ideal drying operation parameters for MWD of mango peels were proposed to be 720 W and 7.5 minutes. Many validation runs were conducted at the achieved optimum parameter values to verify the precision of the forecasts and the applicability of the models. Response Surface Methodology (RSM) responses under ideal conditions were in good agreement with experimental results and had a low relative deviation level of 0.015–0.414. At the optimized condition, TPC (112.088 mg GAE/g), TFC (189.116 mg QE/g), FRAP (15774.380 µg TE/g) and DPPH (9.083 mg GAEAC/g) values were obtained.

Keywords: Mango peel, microwave drying, drying kinetics, antioxidant activity

Determination of vinegar adulteration using a stable carbon isotope analyzer

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Abstract

In this study, a newly developed method which is based on measuring simultaneously the stable carbon isotope ratio for synthetic acetic acid (δ13C acetic acid) in vinegar by CRDS (Cavity Ringdown Spectrometer). The method provided quite precise results and accuracy. With this method, data from 10 different vinegars and were used to evaluate for the adulteration detection. On the basis that all δ13C vinegars are nearly constant, a characteristic pattern of the stable carbon isotope in fruit vinegar was built with the 95% confidence intervals for δ13C value ranged between −25.34 to −27.24. An adulteration detection curve of Δδ13C was proposed based on the results of vinegar and acetic acid samples and confirmed by vinegar spiked with different amounts of synthetic acetic acid. This method could be useful in estimating the blending ratio of adulterated fermented fruit vinegar products. Products containing more than 5% of synthetic acetic acid could be possibly identified.

Keywords: Adulteration, vinegar, stable carbon isotope analyzer, CRDS

Introduction

The realm of isotopes, characterized by atoms sharing identical proton numbers but divergent neutron counts, opens a gateway to profound scientific inquiry. Despite their identical atomic numbers, the nuanced atomic weights and unique nuclear properties of isotopes categorize them into two fundamental groups: the dynamically transformative radioactive isotopes and the steadfast stable isotopes.

As we transitioned into the 21st century, the landscape of analytical precision underwent a paradigm shift with the introduction of diode laser spectroscopy systems. These systems, equipped to discern isotope mixing ratios with unparalleled accuracy, sparked a renaissance in scientific investigation. This technological leap heralded the formation of a global isotope library, a testament to the collaborative efforts in unraveling the mysteries encapsulated within isotopic signatures.

Recent strides in scientific innovation have yielded highly sensitive techniques capable of differentiating minute isotope variations, pushing the boundaries of precision to an astonishing 0.3 ‰. A focal point of this advancement lies in unraveling the intricate isotope changes arising from the diverse photosynthetic pathways of plants. This exploration not only enhances traceability but also establishes a robust framework for detecting adulterations in plant-based products.

The synergy of isotope separation techniques and cavity extinction spectroscopy, epitomized by the Combustion Modulus Isotope Proportioning Cavity Damping Spectroscopy (CM-CRDS), alongside the versatile Isotope Ratio Mass Spectrometry (IRMS) and laser-induced spectrometers like Wavelength-Scanned Cavity Ring-Down Spectroscopy (WS-CRDS), empowers scientists to discern subtle distinctions between C3 and C4 plant groups. This capability proves pivotal in the discernment of potential adulterations within the intricate landscape of food products (Geană et al 2020).

Turning our focus to vinegar, a venerable seasoning celebrated for its ability to bestow a distinctive aroma and sour taste upon culinary creations, we delve into its enigmatic authenticity. Derived from an array of fermentable sources such as wine, molasses, fruits, and grains, vinegar occupies a cherished place in culinary traditions globally (Ozen, 2021). Despite its culinary eminence and multifaceted health benefits, the integrity

of vinegar is jeopardized by potential adulterations, where acetic acid sourced from non-fermentable origins undermines its authenticity.

This study emerges as a beacon in the pursuit of unraveling potential adulterations within commercially available vinegars. By harnessing the analytical prowess of stable carbon isotopes through a Cavity Ring-Down Spectrometer (CRDS), specifically a carbon isotope analyzer, we embark on a journey to fortify the guardianship of vinegar authenticity. Our endeavor not only addresses the paramount concern of consumer health risks associated with potential adulterants but also stands as a testament to our commitment to upholding stringent food regulations in the ever-evolving landscape of culinary craftsmanship.

Material and Method

Plant material

Synthetic acetic acid and 10 different fruit vinegar products were collected from local stores in the city of Adana, Turkey. The fruit vinegars were authentic and obtained from five dominant manufacturers of the Turkish market.

Chemicals

All chemicals and solvents were purchased in high purity grade. Three different brands of glacial acetic acid (WS-Aa1, WS-Aa2, and WS-Aa3) were from Merck (Darmstadt, Germany), VWR (PA, USA), and Macron (PA, USA), respectively. The standard stock solutions of each glacial acetic acid were prepared at 10,000 mg/L in acetone. Stable isotope reference material of IAEA-CH-6 (sucrose, δ13CVPDB: −10.45‰), USGS24 (graphite, δ13CVPDB: −16.05‰), were from international atomic energy agency (IAEA, Vienna, Austria).

Carbon isotope analysis of vinegar

Carbon isotope analysis was carried out with a slight modification to Selli et al. published report (2021). Picarro's Cavity Ring-Down Spectrometer was used to determine the isotope ratios of the honey sample (Figure 1) (CM-CRDS) (G2121-i Picarro Inc. Santa Clara, CA). The vinegar samples were burned in a flash combustion machine during the study. (Picarro Combustion Module) at 980°C. Picarro's Liaison interface was used to gather CO2 from the flash combustion unit. The carrier gas (nitrogen) flow rate was set at 90 ml/min, while the combustion gas (oxygen) flow rate was set to 30 ml/min. Following that, the collected CO2 was automatically delivered into the CRDS sampling chamber for 13C analysis after the mixing time was completed.

Figure 1. Stable carbon isotope analyzer

Results and Discussion

Table 1 presents carbon isotope values (Δ values) for various vinegar samples in comparison to a standard C3 reference. The Δ values indicate the deviation in isotopic composition from the reference.

Table 1. Carbon isotope values of different vinegar damples

	SAMPLE	AVERAGE Δ VALUE FOUND
1	Standard C_3 Reference	-26,61
2	Apple Vinegar	-26,34
3	Grape Vinegar	-26,72
4	Hawthorn Vinegar	-26,08
5	Red wine Vinegar	-27,24
6	White wine vinegar	-27,05
7	Malt vinegar	-25,34
8	White vinegar	-25,91
9	Mulberry vinegar	-26,72
10	Adulterated vinegar with synthetic acetic acid	-31,85
11	Synthetic acetic acid standard	-39,45

These findings underscore the effectiveness of utilizing stable carbon isotope analysis, specifically Continuous Flow Isotope Ratio Mass Spectrometry (CRDS), in discerning the adulteration of vinegar through the addition of synthetic acetic acid. The CRDS results in this study demonstrated a distinct range in carbon isotope ratios for naturally fermented vinegars derived from C3 plants, spanning from -25.34 to -27.24. Notably, this natural variability provides a baseline against which alterations due to adulteration can be identified.

The introduction of synthetic acetic acid into vinegar led to significant shifts in carbon isotope ratios, making the adulterated samples easily distinguishable from their authentic counterparts. Notably, these results align with prior research conducted by Fang et al. (2020), who employed Isotope Ratio Mass Spectrometry (IRMS) to evaluate 14 different vinegar samples. Fang et al. (2020) successfully detected discrepancies between original and inauthentic vinegars by analyzing the carbon isotopic signatures, affirming the reliability and consistency of stable carbon isotope analysis as a robust tool for detecting adulteration in vinegar samples. The method's ability to unveil such subtle compositional changes positions it as a valuable technique in ensuring the integrity and authenticity of vinegar products in the food industry.

In conclusion, the findings from the stable carbon isotope analysis using CRDS present a powerful means of detecting adulteration in vinegar, specifically through the addition of synthetic acetic acid. The distinct carbon isotope ratios observed in naturally fermented vinegars derived from C3 plants provided a reliable baseline for identifying alterations induced by adulteration. The significant shifts in carbon isotope ratios in adulterated samples, as revealed by CRDS, highlight the sensitivity of this analytical technique in discerning subtle compositional changes. These results align with previous research by Fang et al. (2020), reinforcing the credibility of stable carbon isotope analysis, including IRMS, as a consistent and robust method for distinguishing between authentic and adulterated vinegars (El Hawari, et al 2021). As a valuable tool in quality control within the food industry, stable carbon isotope analysis can contribute to safeguarding the authenticity and integrity of vinegar Considering the results, it is clear how simple and quick it is to identify adulterated vinegar by adding synthetic acetic acid when using stable carbon isotope analysis, particularly Cavity Ring-Down Spectroscopy (CRDS). According to CRDS data, naturally occurring vinegars that are made from C3

plants and that undergo conventional fermentation have carbon isotope ratios between -25.34 and -27.24. On the other hand, vinegars tainted with synthetic acetic acid show significant increases in carbon isotope ratios, and the degree of these increases is directly related to the addition rate. This demonstrates the strong ability of CRDS to identify and measure vinegar adulteration, providing a dependable approach to quality management and guaranteeing vinegar authenticity.

Conclusion

Considering the results, it is clear how simple and quick it is to identify adulterated vinegar by adding synthetic acetic acid when using stable carbon isotope analysis, particularly Cavity Ring-Down Spectroscopy (CRDS). According to CRDS data, naturally occurring vinegars that are made from C3 plants and that undergo conventional fermentation have carbon isotope ratios between -25.34 and -27.24. On the other hand, vinegars tainted with synthetic acetic acid show significant increases in carbon isotope ratios, and the degree of these increases is directly related to the addition rate. This demonstrates the strong ability of CRDS to identify and measure vinegar adulteration, providing a dependable approach to quality management and guaranteeing vinegar authenticity.

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Exploring mycelium as a sustainable and alternate protein source for developing iron and vitamin D2 rich low moisture meat analogue

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ABSTRACT

With increasing population and limited natural resources, providing a healthy protein-rich diet is a major challenge especially to vegetarians. Thus, a sustainable alternate protein source is a need. Mycelium is one such novel source of protein that can be grown with less carbon footprint saving the ecology. It has an important advantage in mimicking meat due to its texture similar to meat and can be a good choice showing its future potential in the vegetable meat industry. The present study investigated the potential of mycelium Cv. Pleurotus eryngii for the development of low moisture meat analogue using extrusion technology. Mycelium was cultivated in lab followed by cold-pressing to reduce moisture content to 55% wet basis which was further dried in freezer drier at -50 °C and 5 mbar. The dried powder was discovered to be high in protein (25%), fiber (18%), Fe (8 mg/100g), and vitamin D2 (314.59 mg/g), which was used to prepare a functional meat analogue. Extrusion was carried out with mycelium (30% w/w) replacing pea protein isolate to develop a low moisture meat analogue (LMMA). Mycelium favored expansion ratio (4.14), water holding capacity (2.72 g/g) and oil absorption capacity (1.77 g/g). The texturization index was found to be greater than 1. The microstructure showed a fibrous layered porous structure with lower bulk density and the FTIR spectra revealed intact bonds in amide I region showing their ability against fiber addition. Results from this study showed that mycelium can be used as a novel replacement for the conventional protein source in a plant-based industry providing similar textural and structural properties and decreasing carbon footprint.

Keywords: extrusion, meat analogue, mycelium, vitamin D2.

Near-Infrared spectroscopy quality parameter analysis in wheat from Albania

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ABSTRACT

Wheat (*Triticum aestivum L.*) is one of the staple foods, and its flour-processed products are an essential part of people's diets. Its role in human nutrition and its flexibility to be processed in a wide range of final products (e.g., bread, pastry, cookies, pasta) distinguish wheat from other cereals. Global markets have become increasingly demanding concerning wheat quality. Despite the non-relevance of global crop production, Albania has a long tradition in wheat production. This study's objective was to apply Near Infrared (NIR) spectroscopy for the cereal quality analysis of physicochemical parameters: protein, gluten, starch, sedimentation index and moisture content to whole kernel wheat produced in Albania. NIR spectroscopy is a non-destructive method widely used to predict the organic compounds of grain materials based on electromagnetic wave interactions. Quality control indicators are applicable in wheat breeding, trade, and processing. NIRS advantage consists in test time and cost reduction. Among two pillars - fundamentals and instruments- a third is highly important in NIR spectroscopy - data analysis. Data analysis maps the NIR absorption or transmittance values to the desired sample properties using machine learning. Seventy-five wheat samples were collected during the harvesting season in 2022 from the main agricultural regions: Fieri, Korça, Elbasani, and Tirana. Protein content varied in the interval 9.2-15.1%, with an average of 12.5%; of them, only four samples were below the threshold of 10.5% established by EU Regulation 687/2008. The starch content ranged in the interval 66.8 -72.0%, with an average of 69.6%. The gluten content varied in the interval 18.2-39.2%, with an average of 28.2%. The sedimentation index interval is 19.2-59.0, with an average value of 39.35. Finally, the moisture varied in the interval 9.5-12.2%, with no wheat sample exceeding the threshold max level of 14.5% according to EU regulation.

Keywords: Wheat, quality parameters, protein, gluten, starch, Albania

Near-Infrared spectroscopy quality parameter analysis in the wheat commodity from Albania

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ABSTRACT

Wheat (*Triticum aestivum* L.) is a staple food, and its flour-processed products are an essential part of people's diets. Its role in human nutrition and its flexibility to be processed in a wide range of final products (e.g., bread, pasta, pastry, cookies) distinguish this grain from other cereals. Globalization of markets has become increasingly demanding concerning wheat quality. Despite the non-relevance of global crop production, Albania has a long tradition in grain cultivation, especially wheat production, driven by political reasons in the past.

This study aims to implement low-cost techniques, such as Near-Infrared spectroscopy, on quality analysis of physicochemical parameters: protein, gluten, starch, sedimentation index, and moisture content to the wheat produced in Albania. NIR spectroscopy is a non-destructive method widely used to predict the organic compounds of grain materials based on electromagnetic wave interactions. Quality control indicators are applicable in wheat breeding, trade, and processing. Among two pillars - fundamentals and instruments- a third is critical in NIR spectroscopy - data analysis. Machine learning and data analysis map the NIR absorption or transmittance values to the desired sample properties.

Seventy-five wheat samples were collected during the harvesting season in 2022 from the central agricultural regions: Fieri, Korça, Elbasani, and Tirana. Protein content varied in the interval of 9.2-15.1%, with an average of 12.5%; only four samples were below the threshold (10.5%) established by EU Regulation 687/2008. The starch content ranged in the interval 66.8 -72.0%, with an average of 69.6%; gluten content was 18.2-39.2%, with an average of 28.2%, and the sedimentation index 19.2-59.0, with an average value of 39.35. Finally, according to EU regulation, the moisture varied from 9.5-12.2%, with no wheat sample exceeding the threshold of 14.5%. In conclusion, the quality parameters of wheat produced in 2022 were in accordance with the legislation. NIRS application will help farmers ensure a product of high quality and increase economic profitability.

Keywords: NIR, wheat, protein, gluten, Albania

INTRODUCTION

Wheat grain belongs to the genus Triticum, part of the Poaceae family, which includes other cereals like corn, barley, rice, and rye. They are cultivated for the edible grain seeds, when ground, forms the basis of our diet (Giraldo, Benavente, Manzano-Agugliaro, & Gimenez, 2019). Wheat (*Triticum aestivum* L.) and its flour-processed products are essential to people's diets. It grows everywhere except in tropical regions (Wrigley, Faubion, & Corke, 2016).

The growing population has increased pressure on the global cereal markets, demanding quality control throughout the cereal commodities supply chain. The big three cereal grains (rice, wheat, and maize) have roles as staple foods as primary daily sources of carbohydrate-based energy. Even though there are differences in preferences for cereals among geographical regions of the world, the wheat commodity is essential not only to the European continent but globally (Wrigley, 2016).

The grain is composed of different bran, endosperm, and germ, each with a different composition. Wheat consists of carbohydrates, proteins, and in less amount by fats. Its bran is rich in fibers; the endosperm contains large amounts of protein, carbohydrates, and iron; the germ vitamins and trace minerals, unsaturated and polyunsaturated fats (Javid Iqbal, Shams & Fatima, 2022; Khalid, Hameed & Tahir, 2023).

Gluten proteins make wheat a unique cereal in its suitability for bread production and a dozen other dought uses. The main component of wheat flour is starch, followed by protein, which is essentially the gluten-forming gliadins and glutenins. The fat content is low. Wheat is a significant source of energy, fiber, vitamins, and minerals in our diet. Wheat is unique among the food grains. It contains storage proteins that form a cohesive dough with extensibility and elasticity to allow the growth of gas bubbles (generally air and carbon dioxide) and to allow the retention of the bubble structure throughout the bread-baking process (Wrigley, 2016).

On average, world wheat production in 2021 reached about 900 million tonnes, grown on some 244 million hectares, with a world average yield of 3.71 tonnes /ha (FAOSTAT, 2022). In the European Union, more than half of cereals grown are wheat. Maize and wheat are the main grains planted in Albania. Their cultivation is present throughout the country but is produced mainly in Fieri, Elbasani, Korça, and Tirana districts. In 2021, wheat production reached 225 thousand tons.

Table 1. Production of field crops in Albania during the harvesting year 2021 (tonnes).

No.	Prefecture	Cereals	Wheat	Maize	Rye	Barley	Oats
1	Berati	32953	11636	17190	-	223	3904
2	Dibra	47266	7588	38516	632	302	227
3	Durrësi	41333	10404	29101	-	36	1792
4	Elbasani	98249	35297	57142	271	198	5341
5	Fieri	168386	62886	88517	-	4939	12044
6	Gjirokastër	16979	6327	8130	-	-	2522
7	Korça	85094	45950	26783	1616	7983	2762
8	Kukësi	14586	2010	11271	758	-	547
9	Lezha	33138	11131	22007	-	-	-
10	Shkodër	52793	5105	47688	-	-	-
11	Tirana	46434	17558	25943	16	1409	1508
12	Vlora	54144	9280	41982	-	220	2662
	Total	**691353**	**225171**	**414271**	**3293**	**15310**	**33309**

Source: 1 Ministry of Agriculture and Rural Development of Albania (INSTAT, 2022).

The wheat flour quality and safety are of public concern since it is related to the flour products' quality and human health (Zhang et al., 2022). Application of Near-Infrared Spectroscopy (NIRS) in grain commodities comprises many quality and quantity parameters. Moisture and protein content are the most widely used and have achieved worldwide adoption due to their economic importance and the accuracy of the respective NIR models approach. Other NIR applications involve macroconstituent analysis (starch, oil), essential constituents (amino acids, beta-glucans, dietary fibers, amylose), quality control during processing (hardness, dough development, wet milling), grading and classification (color class, vitreousness, insect), food quality (mycotoxins, contaminants, ergot bodies) (Johnson, 2020; Delwiche, 2021). The NIRS technique is advantageous due to its numerous advantages. It reduces the test time and costs. It does not require chemicals and sample preparation, making it superior to traditional chemical analytical methods, especially in industrial quality control and process monitoring applications.

American Society for Testing and Materials (ASTM) has defined near-infrared light as part of the electromagnetic spectrum in the range of 780–2500 nm, between the visible and mid-infrared light spectrum (Schustera, Huenb, & Scherf, 2023). NIR spectroscopy is a technique that applies the NIR portion of the electromagnetic spectrum and can provide complex structural information related to the vibration behavior of chemical bonds (Kamal & Karoui, 2015). NIR spectra present the overtones and combination of hydrogen-containing C–H, O–H, and N–H groups, which are the primary structural components of organic compounds, such as water, lipids, and proteins (Schustera, Huenb, & Scherf, 2023).

The measurement proces consists of the following steps: (1) spectral data acquisition; (2) data pre-processing eliminate noises; (3) building calibration models obtained by suitable reference methods; and (4) model validation using another set of samples without the calibration set (Cen & He, 2007). Among two pillars - fundamentals and instruments- a third is highly important in NIR spectroscopy - data analysis. Using machine learning, data analysis maps the NIR absorption or transmittance values to the desired sample properties.

The NIR spectroscopy algorithms used to "interpret" optical data for absorbing samples may be explained as different approaches to relating sample absorbance (A) at specific wavelengths to analyte concentrations via Beer's law.

So the multi-regression equation commonly used for calibration is:

$$Y = Bo + Bi(-\log Ri)N + E$$

Where:
Y = percent concentration of the absorber
Bo = intercept from regression
Bi = regression coefficient
i = index of the wavelength used and its corresponding reflectance (Ri)
N = total number of wavelengths used in regression
E = random error

MATERIALS AND METHODS

Wheat samples

A total of 75 wheat samples from the central producing regions of Albania: Fieri, Elbasani, and Korca, were collected during the summer of 2022, directly after harvesting. The wheat samples were prepared according to the sampling procedure and placed in bags. Finally were transferred to laboratory premises and kept at 7oC in a dark place until analysis.

The overall methodology used in the study

The objective of this study was NIRS application for analysis of quality parameters: protein, gluten, starch, and sedimentation index to whole kernel wheat produced in Albania. FOSS infrared apparatus for analyzing the physicochemical parameters of cereals.

Infratec ™ 1241 is a device that analyzes whole grains using near-infrared technology to test multiple parameters—Standard EN 15948:2015 - Cereals - Determination of moisture and protein - Method using Near-Infrared-Spectroscopy in whole kernels. The parameters analyzed are moisture, protein, gluten, sediment, starch, and fat. It consists of a Hopper where a sample of at least 400g is placed. Transmission mode measurements are made at a lower wavelength of 570 - 1050 nm, while primary information for reflectance measurements is obtained between 1100 - 2500 nm. The higher level of light energy in the lower range allows deeper penetration into the interior of the grain and not only the surface but also the interior of the seed kernel.

RESULTS AND DISCUSSIONS

NIRS is a non-destructive and rapid technique which is increasingly for food quality evaluation in recent years. It provides us with more information to research the quality of food products (Cen & He, 2007). One of the oldest and most important applications of NIRS in the food and agriculture sector is for measuring protein content in grains, especially wheat. In recent years, spectral analysis technology has been used for testing seed quality, such as determining protein, wet gluten, moisture, ash, and sedimentation in wheat flour (Chen et al., 2021). The NIRS technique for wheat quality control can be applied in two different ways: as an analytical method for accurate and rapid determination of composition in trade and as a screening method in wheat breeding and processing (Williams, 2007). Each NIRS instrument is designed to contain a sample compartment, light source, wavelength selection system, and detector. Measuring wheat protein content has succeeded due to strong and broad absorption by the N–H bonds in the NIR spectral region. The importance of accuracy is so high because premiums of 1.30–1.50$ are paid for increments of 0.1 % in protein content per ton (EC 687/2008). In reflectance, whole grain samples were scanned in a NIR monochromator instrument (400-2500 nm). Partial least squares (PLS) were used to develop calibration equations for the quality characteristics of whole wheat (Pojic & Mastilovic, 2013).

As one of the various technologies for evaluating food quality, the NIRS technique has its advantage in food analysis. The spectral measurement for one sample may finish in 1 min. This method is less expensive compared with tradicional methods because of no solvent and expensive instruments (Cen & He, 2007).

Accordingly, the primary goals of this research are as follows: (1) determine protein, starch, and moisture content in wheat flour using traditional chemical methods and obtain NIR-HSI image information.

NIRS was particularly useful due to the numerous advantages it provides. The application of the NIRS technique significantly reduces the test time and costs. It does not require chemicals and sample preparation, making it superior to traditional chemical analytical methods, especially in industrial quality control and process monitoring applications. In the case of the cereal-based food and feed industry, benchtop NIRS systems with calibrations based on single-cereal species are routinely utilized.

The prerequisite for using the NIRS method is an appropriate calibration model developed to relate the compound (or property) of interest to the sample's spectral data. NIRS calibration model development is very demanding due to the complex composition of analyzed samples, resulting in severe overlapping of interfering bands representing the component (s) (or properties) of interest. NIRS devices, chemometrics techniques, and computer technology have contributed to developing the NIRS technique currently used for testing a wide range of foodstuffs.

Gluten composition in wheat flour is an important quality parameter because it strongly correlates to baking quality. Protein quantity is often insufficient to predict baking quality because different baking quality was observed for flours with comparable protein content but different gluten compositions (Schuster, Huen, & Scherf, 2023).

The quality and quantity (normal variation ranges 8–16%) of wheat flour protein affects the dough properties, consecutively the quality of the final product. The main components of wheat flour are protein (about 10–12%) and starch (about 70–75%), while the secondary components are polysaccharides (about 2–3%) and lipids (about 2%) (Chen, 2021). The proteins form a three-dimensional structure during dough mixing, giving elasticity, helping it rise and keep its shape, and providing strength and a chewy texture to the baked goods.

Table 2. Chemico-physical parameters measured in the wheat commodity produced during 2022 (%).

No.	Proteins	Humidity	Starch	Gluten	Sedimentation index
Min	9.20	9.50	66.80	18.20	19.20
Max	15.10	12.20	72.00	39.20	59.00
Average	12.54±1.27	10.86±0.53	69.59±1.21	28.17±4.34	39.35±8.62

This study's objective was to apply NIR spectroscopy for the cereal quality analysis of physicochemical parameters: protein, gluten, starch, sedimentation index, and moisture, in wheat grain produced in Albania.

Seventy-five wheat samples were collected during the harvesting season in 2022. Protein content varied in the interval 9.2-15.1%, with an average of 12.5%; of them, only four samples were below the threshold (10.5%) (EU Regulation 687/2008). The starch content ranged in the interval 66.8 -72.0%, with an average of 69.6%.

The gluten content varied in the interval 18.2-39.2%, with an average of 28.2%. The minimum gluten content of wheat flour should be about 24 % (wet) (Kaushik, Kumar, Sihag & Ray, 2015; Baeten et al., 2019). Only four samples, or 5.3% of analyzed wheat samples, were found under 24%, showing the very good quality of the wheat produced during 2022.

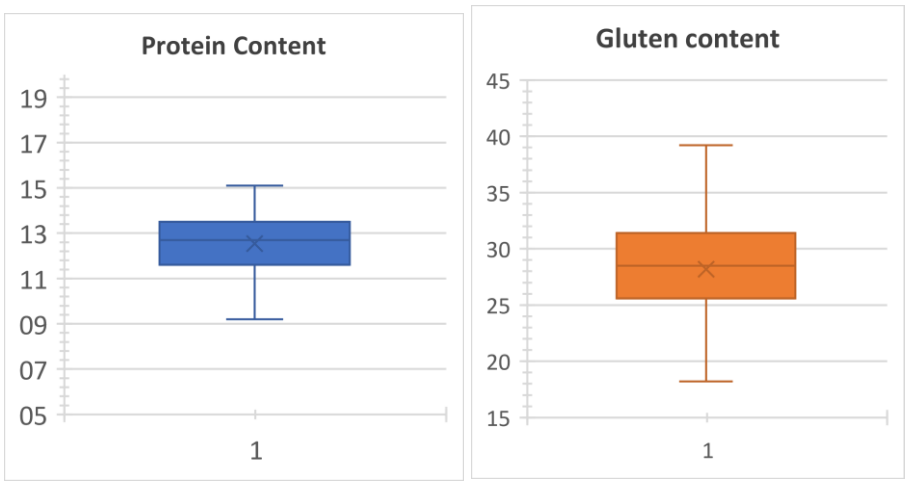

Figure 2. Protein and gluten content in wheat samples (%).

The sedimentation index interval is 19.2-59.0%, with an average value of 39.35%. The sedimentation test is a physicochemical test that helps provide information on wheat flour's baking quality. The sedimentation value, according to Zeleny (Zeleny value), describes the degree of sedimentation of flour suspended in a lactic acid solution, referring to a standard time interval. This is taken as a measure of the quality of baking. A higher gluten content and a better gluten quality give rise to slower sedimentation and higher Zeleny test values (Hruskoca & Famera, 2003).

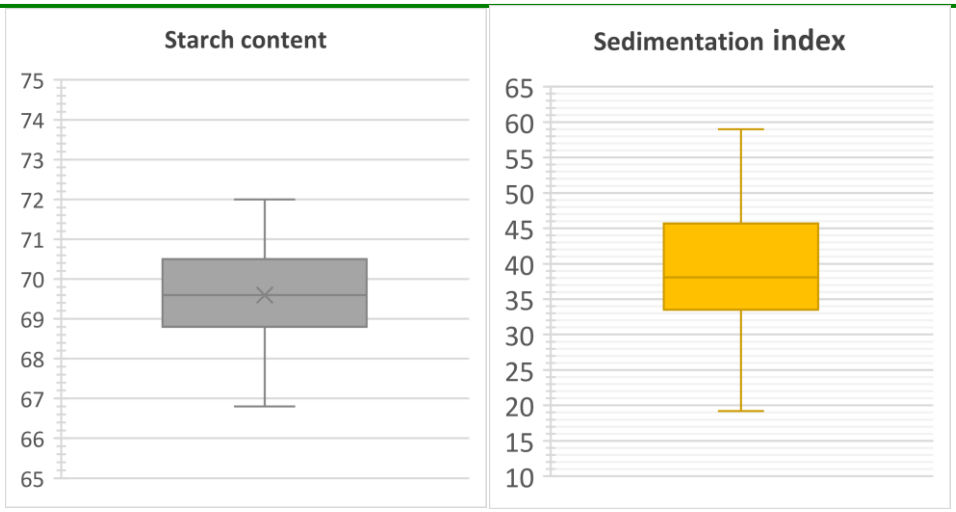

Figure 3. Starch content and sedimentation index of wheat samples.

The content of moisture is critical for the storage of flour. High moisture content in flour promotes infestation with mold and worms. According to EU regulation, the moisture varied at 9.5-12.2%, with no wheat sample exceeding the threshold max level of 14.5%. Average values of protein content were found to be 12.54±1.27%, with maximum values of 15.1%. One of the critical concerns regarding food safety worldwide is the mycotoxin contamination of cereals. Regulated and non-regulated mycotoxin contamination is a continuous threat to the world population due to the importance that cereals like wheat and maize are stapled foods. Chronic exposure may lead to several adverse effects and target toxicity organs. Issues on mycotoxin contamination have been in focus in Albania regarding cereals contamination (Topi et al., 2021; Topi et al., 2017). Using cereals and their by-products as feed may result in animal health threats and human exposure to dairy products (Muharremi et al., 2021; Topi et al., 2022). Still, other constituents, such as aflatoxin in corn or deoxynivalenol in wheat, have received the attention of NIR practitioners despite the concentrations of their natural occurrence in bulk lots, at sub-ppm levels, being below the detection limits of NIR reflection or transmission spectroscopy (Levasseur-Garcia, 2018; Topi et al., 2022).

CONCLUSIONS

Quality control of wheat and wheat flour is economically important for consumer protection. NIR spectroscopy application for cereal quality analysis indicates that wheat commodity production in Albania is of high quality. Climate change impact both food security and food safety of cereal production. Implementing low-cost and portable techniques such as NIRS will support farmers in cereal production and increase awareness of the importance of quality parameters in the economic aspect.

ACKNOWLEDGMENT

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Microbial Evaluation of Fermented Beetroot Juice Produced by Probiotic *Lacticaseibacillus Paracasei*

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ABSTRACT

Probiotic products have a significant proportion in functional food market, and research on the use of fruits and vegetables instead of dairy products in the production of probiotic products is increasing due to many factors. Red beetroot juice can be produced spontaneously or by probiotic bacteria. It is important to determine a product-specific pasteurization parameter to ensure that the product is microbially safe. Red beetroot is a very valuable plant due to its phenolic components and betalains, which have many important effects on health. The aim of this study is to produce a probiotic drink from red beetroot juice, which is so important for health, and to enable consumers to benefit more from red beetroot. In this study, the red beetroot juice samples produced by probiotic strain *Lacticaseibacillus paracasei* 431®, 17 different runs were created with the Box Behnken experimental design in Responce Surface Methodology. As independent variables; temperature (60-80°C), time (10-30 min.), and fermentation temperature (24-36°C) were selected. To demonstrate the effectiveness of the pasteurization process, total yeast and mold (TYM), and total mesophilic bacteria (TMB) were determined right after pasteurization and before fermentation. The results showed that; before fermentation, TYM and TMB counts of the samples were 0.50-2.87 log cfu/mL and 0.35-4.12 log cfu/mL, respectively. According to the ANOVA test results, models were significant, and also temperature and time were found to be significant for both responses ($p < 0.05$). After fermentation, TYM, TMB and total lactic acid bacteria (LAB) counts of the samples ranged between 8.29-9.12 log cfu/mL, 8.50-9.25 log cfu/mL, and 8.17-9.01 cfu/mL, respectively. Although differences were determined between the microbial loads of the samples at the beginning of the fermentation, the effect of the models determined were found insignificant at the end of the fermentation ($p > 0.05$).

Aroma components of *Glycyrrhiza glabra* molasses

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ABSTRACT

Licorice (*Glycyrrhiza glabra*), a perennial shrub plant belonging to the Legume family, thrives in diverse environments such as cultivated fields, alluvial river valleys, and dunes. Renowned for its pharmaceutical properties, the licorice plant holds significance in various applications. In the course of the present study, volatile components of licorice were meticulously extracted using liquid-liquid extraction methods and subsequently analyzed through gas chromatography-mass spectrometry (GC-MS). The GC-MS results revealed the identification and quantification of a total of 64 volatile compounds within the licorice sample. Notably, a diverse range of chemical classes, including alcohols, ketones, volatile phenols, pyrazines, aldehydes, acids, furans, pyrroles, terpenes, pyridines, and pyranones, were found in the licorice molasses sample. This comprehensive analysis provides a detailed insight into the complex and multifaceted aroma profile of licorice, highlighting the diverse array of volatile compounds present. The identification and quantification of these compounds contribute to our understanding of licorice flavor characteristics, offering valuable information for various industries, including pharmaceuticals, food, and cosmetics.

Keywords: Licorice, GC-MS, volatile compounds, liquid-liquid extraction

INTRODUCTION

Licorice, recognized as a natural spreading plant species, boasts an herbaceous root that flourishes in various regions across the globe. While extensively used in America and Europe, its utilization in Turkey remains relatively limited and nearly nonexistent. Notably, licorice finds prominence in Asia, with Pakistan, India, China, Iran, Turkmenistan, Georgia, Uzbekistan, and Kazakhstan emerging as major producers of licorice root (Khalesi, 2015).

According to 2019 data from the Food and Agriculture Organization (FAO), global licorice root production reached an impressive 9,924,808 tons. Turkey assumes a significant role as both a producer and exporter of licorice root. Renowned for its antioxidant, antibacterial, anticancer, and antiviral effects, the licorice plant has been employed in the treatment of various ailments in both modern and traditional medicine, a tradition spanning from ancient times to the present day (Xu et al., 2013; Qiao et al., 2015).

The roots of the licorice plant have gained attention for their richness in bioactive compounds, presenting numerous health benefits, as highlighted by Pastorina et al. (2018). This multifaceted plant finds applications in the realms of health, food, and cosmetics.

Within this context, the aromatic compounds of licorice root molasses emerge as pivotal quality factors, significantly influencing the sensory properties of the product. The primary objective of our study is to elucidate and identify these aroma compounds, given their importance in the fields of health, cosmetics, and food. This exploration aims to contribute to the broader understanding of licorice-derived products, furthering their potential applications and value in diverse industries.

MATERIALS AND METHODS

Licorice roots obtained from Kahramanmaraş province were separated from their stems and garbage, washed and dried. Then, licorice root juices were obtained by brewing with water according to the temperature, duration and solid/liquid ratios under the process variables specified. After brewing, the licorice root juices were squeezed using a juice juicer and the resulting waters were filtered using coarse filter papers before the analyses.

Aroma Analysis

In the analysis of aroma, the liquid-liquid extraction method emerged as a pivotal technique. A specific quantity of licorice molasses, an internal standard, and dichloromethane underwent meticulous mixing for a duration of 2 hours within a cold environment under a nitrogen gas atmosphere. Following this, the mixture underwent centrifugation, facilitating phase separation in a specialized separation funnel. The ensuing solvent phase underwent evaporation, concentrating the aroma mixture, which was subsequently transferred to an evaporation flask. From there, it was carefully dispensed into vials and precisely injected into the GC-MS device for further analysis.

The aromatic profile revealed the prevalence of various compound classes, with alcohols, ketones, volatile phenols, pyrazines, aldehydes, acids, and furans standing out as significant constituents of the aroma compounds. Notably, these compounds exhibited consistent presence in licorice samples across diverse countries, as corroborated by previous studies (Farag and Wessjohann, 2012; Gyawali et al., 2008; Russo et al., 2014; Wagner et al., 2016). This collective body of research underscores the widespread nature of these aroma compounds in licorice, contributing valuable insights to our understanding of its olfactory characteristics on an international scale.

GC-FID and GC-MS Conditions

The quantity determination, identification, and determination of aroma-active compounds were simultaneously performed using the Shimadzu QP2020 gas chromatography coupled with the Shimadzu QP2020 mass spectrometry and olfactometry. In this system, the column effluent is divided into three equal parts with the help of a specialized separator (Dean switch-Agilent): the first part goes to the Flame Ionization Detector (FID), the second part to the Mass Spectrometry Detector (MSD), and the third part to the olfactometry.

Consequently, the simultaneous quantification, identification, and sensory analysis enhance the precision of the analysis.

In the determination of aroma compound quantities, a flame ionization detector (FID) gas chromatography system, namely the Shimadzu QP2020, was employed. The separation of aroma compounds was achieved using a DB-WAX capillary column (30 m × 0.25 mm × 0.5 m). The injector temperature was set at 220 °C, the detector temperature at 250 °C, and the column temperature was programmed to start at 60 °C for 3 minutes, followed by an increase of 2 °C per minute until reaching 220 °C. Subsequently, the temperature was further increased at a rate of 3 °C per minute, reaching 245 °C, and maintained at this temperature for 20 minutes. The injected volume into the system was 3 µl. Helium was used as the carrier gas with a flow rate of 1.5 ml per minute. Both the detector and injector temperatures were maintained at 250°C (Le Guen et al., 2000).

In the characterization of aroma compounds, a mass spectrometer, specifically the Shimadzu QP2020, coupled with gas chromatography was employed. The injector type and temperature program matched the conditions used in gas chromatography. Helium was used as the carrier gas at a flow rate of 1.5 ml/min. The mass spectrometer operated with an ionization energy of 70 eV, an ion source temperature of 250 °C, and a quadrupole temperature of 120 °C. Scanning was performed at one-second intervals over the mass-to-charge ratio (m/e) range of 29-350. Compound identification of peaks was carried out by injecting standard solutions for compounds with known standards and comparing the mass spectra of compounds without standards with those in the aroma compound libraries (Wiley 9.0, NIST-11, and Flavor.2L) stored in the computer memory. Following peak identification, the concentrations of aroma compounds were calculated using the internal standard method (Selli et al., 2008). Each analysis was conducted in triplicate.

RESULTS AND DISCUSSIONS

In the process of identifying aroma substances, a comprehensive approach was undertaken, leveraging a library that incorporated mass spectroscopy (MS), aroma standards (Std), and retention index (LRI) values. This amalgamation of analytical tools facilitated a thorough and accurate identification of aroma compounds present in the licorice root molasses sample. The utilized library, encompassing data from mass spectroscopy, aroma standards, and retention indices, ensured a robust and reliable identification process. A total of 64 distinct aroma compounds were successfully determined in the licorice root molasses sample, showcasing the richness and complexity of its aromatic profile. The application of advanced analytical techniques and a diverse library of standards underscores the commitment to precision in characterizing the intricate flavor constituents of licorice root molasses. These findings contribute not only to the specific understanding of licorice aroma but also advance the broader field of flavor science, offering valuable insights for the continued exploration and enhancement of flavor profiles in various food products.

Alcohols

In our comprehensive analysis of alcohol compounds, two particularly noteworthy constituents within the aromatic alcohol group emerged as dominant in terms of quantity: benzyl alcohol and 2-phenylethanol. Among these, 2-phenylethanol holds particular significance as a key aromatic alcohol renowned for imparting a distinct rosy fragrance. This compound, prevalent in a variety of food items, plays a crucial role in shaping the overall sensory experience.

The prominence of benzyl alcohol and 2-phenylethanol underscores their prevalence in the licorice molasses under investigation. As aromatic alcohols, their distinctive olfactory characteristics contribute significantly to the overall aroma profile of the licorice. The identification and quantification of these compounds not only provide insights into the quantitative distribution within the sample but also shed light on the sensory attributes associated with licorice products.

The aromatic richness brought about by benzyl alcohol and 2-phenylethanol highlights their potential impact on the flavor composition of licorice-based products. Understanding the specific aromatic contributions of these compounds contributes to the broader comprehension of licorice flavor profiles, enabling further exploration and refinement in the food and beverage industry.

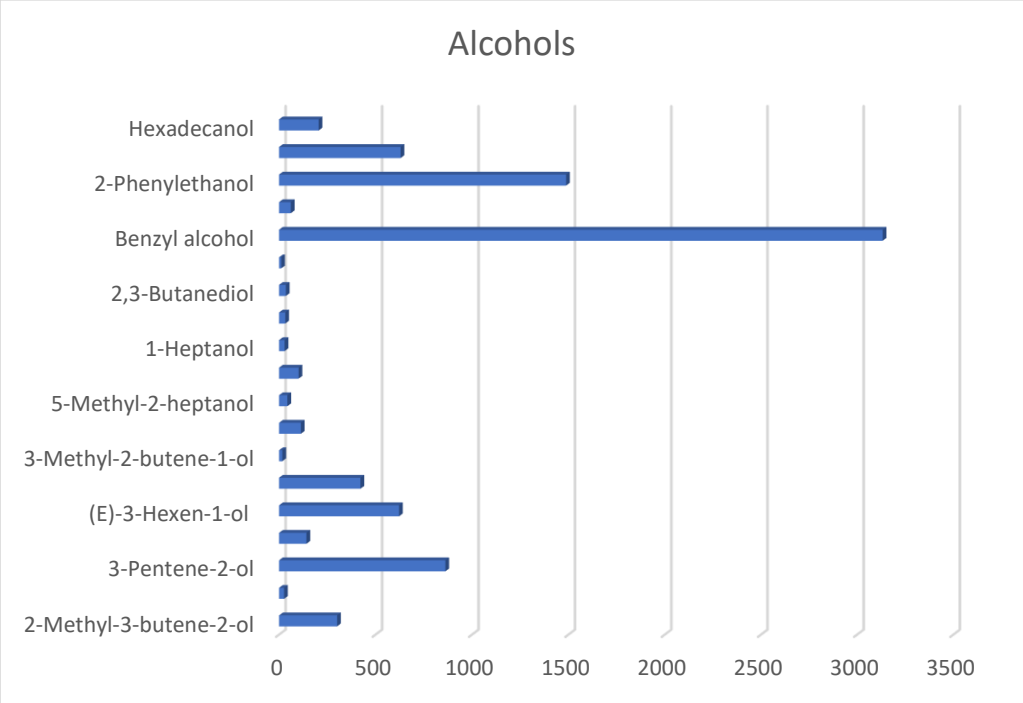

Figure 1. Alcohol compounds in licorice molasses

Ketones

In the exploration of licorice root molasses samples, our analysis unveiled the presence of a total of six distinct ketone compounds. Among these, one compound stood out as the most dominant in terms of quantity acetovanillone, with a concentration of 668 µg/L.

The identification of multiple ketone compounds adds a layer of complexity to the aromatic profile of licorice root molasses, reflecting the diverse array of flavor constituents present. Acetovanillone, in particular, emerges as a key player within this chemical class. Known for its distinctive aromatic characteristics, acetovanillone contributes significantly to the overall sensory experience of licorice-based products. This finding shed light on the intricate composition of ketone compounds in licorice, offering valuable insights for flavor characterization and quality assessment. The quantification of acetovanillone, with its notable concentration, underscores its potential impact on shaping the unique flavor profile of licorice root molasses. This information holds practical significance for industries involved in the production and formulation of licorice-flavored products, facilitating informed decisions in enhancing and optimizing flavor attributes.

Table 2. Ketone compounds in licorice molasses

No	LRI	Compound Name	Concentration (µg/L)
		Ketones	
1	1180	2-heptanone	14.1±0.7
2	1238	3-hydroxy-3-methyl-2-butanone	107±6.2
3	1287	3-hydroxy-2-butanone	65.7±0.9
4	1342	6-methyl-5-hepten-2-one	46.4±1.7
5	1439	1-hydroxy-2-pentane	5.9±0.1
6	2620	Asetovanillone	668±9.7

Volatile phenols

In the examination of licorice root molasses samples, our analysis revealed the presence of a total of seven volatile phenol compounds, namely guaiacol, phenol, 4-ethyl guaiacol, m-guaiacol, 4-vinyl guaiacol, isoeugenol, and 4-hydroxy-3-methoxybenzaldehyde (vanillin). Notably, among these volatile phenol compounds identified in this study, vanillin emerged as the most dominant in terms of quantity.
The recognition of multiple volatile phenol compounds adds depth to the aromatic complexity of licorice root molasses, highlighting the diverse range of flavor constituents present. Vanillin, in particular, takes center stage within this chemical class, showcasing its substantial contribution to the overall sensory profile of licorice-based products. This study's findings align with previous research conducted by Wagner et al. (2016), who emphasized the significance of 4-hydroxy-3-methoxybenzaldehyde (vanillin) as one of the most crucial aroma compounds in fresh licorice root samples. The prevalence of vanillin in licorice root molasses underscores its importance in defining the characteristic aroma associated with licorice products, providing valuable insights for flavor analysis, quality assessment, and the formulation of licorice-flavored goods.

Table 3. Volatile phenol compounds in licorice molasses.

No	LRI	Compound Name	Concentration (µg/L)
		Phenols	
1	1823	Guaiakol	225±0.6
2	1973	Phenol	429±4.2
3	2008	4-Ethylguaiacol	573±10.8
4	2086	3-Methoxyphenol	980±30.6
5	2183	4-Vinylphenol	579±0.1
6	2266	Isoogenol	353±17.6
7	2566	4-hydroxy-3-methoxybenzaldehyde	3947±64.6

Pyrazines

In the examination of licorice root molasses samples, our analysis revealed the presence of a total of seven volatile phenol compounds, namely guaiacol, phenol, 4-ethyl guaiacol, m-guaiacol, 4-vinyl guaiacol, isoeugenol, and 4-hydroxy-3-methoxybenzaldehyde (vanillin). Notably, among these volatile phenol compounds identified in this study, vanillin emerged as the most dominant in terms of quantity. The recognition of multiple volatile phenol compounds adds depth to the aromatic complexity of licorice root molasses, highlighting the diverse range of flavor constituents present. Vanillin, in particular, takes center stage within this chemical class, showcasing its substantial contribution to the overall sensory profile of licorice-based products. This study's findings align with previous research conducted by Wagner et al. (2016), who emphasized the significance of 4-hydroxy-3-methoxybenzaldehyde (vanillin) as one of the most crucial aroma compounds in fresh licorice root samples. The prevalence of vanillin in licorice root molasses underscores its importance in defining the characteristic aroma associated with licorice products, providing valuable insights for flavor analysis, quality assessment, and the formulation of licorice-flavored goods.

Table 4. Pyrazine compounds in licorice molasses

No	LRI	Compound Name	Concentration (µg/L)
		Pyrazines	
1	1276	Methylpyrazine	580±2.2
2	1333	2,5-Dimethylpyrazine	758±1.2
3	1346	2,3-Dimethylpyrazine	1274±0.1
4	1402	2-Ethyl-5-methylpyrazine	335±7.0
5	1425	2,5-Diethylpyrazine	596±10.8
6	1464	2-Ethyl-3,5-dimethyl pyrazine	4.4±0.1

Aldehydes

Within the spectrum of aldehyde compounds identified in our analysis of licorice root molasses samples, two compounds, benzaldehyde (1004 µg/L) and 4-methoxy-benzaldehyde (426 µg/L), emerged as prominent contributors to the total aldehyde content. Benzaldehyde, in particular, holds significance as a key compound known for imparting an almond-like aroma to various foods. Its prevalence in licorice root molasses aligns with prior studies that have detected benzaldehyde in different licorice samples (Wagner et al., 2016; Tanaka et al., 2008).

The notable quantities of benzaldehyde and 4-methoxy-benzaldehyde underscore their impact on shaping the overall aroma profile of licorice root molasses. The almond-like fragrance associated with benzaldehyde adds a distinctive sensory dimension to the flavor composition of licorice-based products. The inclusion of these aldehyde compounds contributes to the nuanced and complex nature of licorice flavor, providing valuable insights for both flavor analysis and the formulation of licorice-flavored goods. These findings further enhance our understanding of the aromatic intricacies within licorice root molasses, contributing to the broader knowledge base in flavor science.

Table 5. Aldehyde compounds in licorice molasses

No	LRI	Compound Name	Concentration (µg/L)
		Aldehydes	
1	1048	Hexanal	169±7.8
2	1076	2-Methyl-2-butenal	66.8±0.5
3	1212	3-Methyl-2-butenal	75.5±1.1
4	1515	Benzaldehyde	1004±7.7
5	1988	4-Methoxy-benzaldehyde	426±0.8

Acids

Owing to the high detection threshold values associated with acids, their influence on the distinctive aroma of foods is somewhat constrained. In our analysis of liquorice root molasses samples, a total of seven different acid compounds were identified. Notably, among these compounds, hexanoic acid exhibited the highest concentration, followed by octanoic and pentanoic acid. While acids may have a limited impact on aroma due to their high threshold values, their presence in licorice root molasses adds a layer of complexity to the overall flavor composition. The varying concentrations of hexanoic, octanoic, and pentanoic acids contribute distinct acidic notes to the sensory profile. Understanding the interplay of these acids in licorice flavor provides valuable insights for flavor scientists and food technologists, aiding in the formulation and enhancement of licorice-flavored products.

This comprehensive analysis expands our understanding of the role of acids in the aromatic profile of licorice root molasses, contributing to the broader knowledge base in the field of flavor science and sensory evaluation of food products.

Table 6. Acid compounds in licorice molasses

No	LRI	Compound Name	Concentration (µg/L)
		Acids	
1	1415	Acetic acid	105±1.8
2	1685	Pentanoic acid	869±10.1
3	1744	(E)-2-Butenoic acid	83.8±4.4
4	1816	Hexanoic acid	1345±2.1
5	2022	Octanoic acid	1067±11.7
6	2132	Nonanoic acid	352±12.5
7	2899	Hexadecanoic acid	125.6±5.3

Furans

Our examination of this specific chemical group, we identified a total of four distinct compounds, namely 2-pentyl furan, 5,5-dimethyl-2(5H)-furanone, 2-furanmethanol, and 4-methyl-5H-furan-2-one. Each of these compounds plays a unique role in contributing to the overall aromatic complexity of the sample. 2-pentyl furan introduces a characteristic note, adding a specific nuance to the flavor profile. 5,5-dimethyl-2(5H)-furanone, with its distinctive molecular structure, contributes to the sensory richness of the licorice root molasses. Additionally, 2-furanmethanol and 4-methyl-5H-furan-2-one each bring their own aromatic qualities to the mix, enhancing the overall flavor experience.

This diverse array of compounds within this chemical group exemplifies the intricate nature of licorice root molasses. The identification and understanding of these specific components contribute significantly to our knowledge of the complex flavor matrix within licorice products. Such insights are invaluable for researchers, flavor scientists, and professionals in the food industry, aiding in the formulation and optimization of licorice-flavored products.

Table 7. Furan compounds in licorice molasses

No	LRI	Compound Name	Concentration (µg/L)
		Furans	
1	1224	2-Pentylfuran	954±53.2
2	1583	5,5-Dimethyl-2(5H)-furanone	1258±51.4
3	1640	2-Furanmethanol	144±5.6
4	1909	4-Methyl-5H-furan-2-one	232±14.7

Pyrroles

These compounds, initially discovered in coffee studies, have since been identified in a wide range of foods, including roasted almonds, cooked asparagus, roasted malt, fried beef, beer, cakes, chocolate, hard-boiled eggs, popcorn, soy sauce, corn chips, and tea. The exploration of pyrroles in various food items reflects their widespread presence in diverse culinary contexts. Our investigation into licorice molasses samples revealed the presence of a total of five pyrrole compounds: 1-methyl-2-pyrrolidone, 3,4-dimethyl-3-pyrroline-2-one, 2-acetyl pyrrole, pyrrole-2-carboxaldehyde, and 2-pyrrolidinone. Each of these compounds contributes to the intricate aromatic profile of licorice root molasses, adding distinctive notes that enhance the overall flavor experience.

The identification of pyrrole compounds in licorice molasses expands our understanding of the flavor composition in licorice products. These findings not only contribute to the knowledge of pyrroles in the context of licorice but also provide valuable insights for the broader field of flavor science and the sensory evaluation of diverse food products.

Table 8. Pyrroles compounds in licorice molasses

No	LRI	Compound Name	Concentration (µg/L)
		Pyrolles	
1	1678	1-Methyl-2-pyrrolidone	954±53.2
2	1996	3,4-Dimethyl-3-pyrroline-2-one	1258±51.4
3	2002	2-Acetylpyrrole	144±5.6
4	2006	Pyrrole-2-carboxaldehyde	232±14.7
5	2017	2-Pyrrolidinone	6315±42.5

CONCLUSION

In conclusion, the study delves into the intricate world of licorice (*Glycyrrhiza glabra*), a perennial shrub with significant pharmaceutical properties. Analyzing the volatile components through liquid-liquid extraction and gas chromatography-mass spectrometry (GC-MS), a total of 64 volatile compounds were identified and quantified in licorice molasses. The diverse chemical classes, including alcohols, ketones, volatile phenols, pyrazines, aldehydes, acids, furans, pyrroles, terpenes, pyridines, and pyranones, underscore the complexity and richness of licorice's aroma profile. This research not only sheds light on the underexplored licorice landscape in Turkey but also contributes valuable insights for various industries, including health, food, and cosmetics, harnessing the potential of licorice-derived products in diverse applications.

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Fermentation of bergamot (*Citrus bergamia*) fruit with *Lactobacillus plantarum*: phenolic compounds, antioxidant activity and prebiotic properties

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ABSTRACT

Studies focusing on the limited cultivation and restricted use of bergamot in food have drawn attention, leading to the exploration of alternative utilization methods. This fruit contains 61 identified metabolites that offer various benefits to human health. This study was conducted to uncover the potential use of bergamot as a fermented food or dietary supplement. The outer skin of bergamot was peeled, leaving the albedo intact, and the entire fruit was homogenized. It was then fermented with *Lactobacillus plantarum* strain for six days. Samples were taken on days 0, 2, 3, 4, and 6 to analyze the viable bacterial count, antioxidant activity using the DPPH and ABTS methods, phenolic compounds using LC-DAD-ESI-MS/MS, and total phenolic content using the Folin-Ciocalteu method. The results were compared with the control group.

On the 6th day of fermentation, the total bacterial count was determined as $5.3x10^{10}$ cfu/mL, pH value was 3.7, and the total phenolic compound content increased to 21.74 GAE/mL compared to the control group (20.78 GAE/mL) and day 0 (20.17 GAE/mL). The antioxidant activity measured by ABTS and DPPH methods increased from 8.56 and 3.25 mM Trolox/L on day 0 to 9.72 and 3.55 mM Trolox/L, respectively. In the control group, the antioxidant capacity decreased to 7.63 and 2.8 mM Trolox/L, according to the ABTS and DPPH methods, respectively. The highest concentrations of phenolic compounds were observed on the 4th day of fermentation. The concentrations of Neoeriocitrin, Naringin, and Neoeriocitrin-di-oxalate increased on the 4th day to 297.23, 160.37, and 55.86 µg/L, respectively, compared to day 0 and the control group. Neohesperidin and Neohesperidin-di-oxalate remained unchanged, while the concentration of melitidin decreased in both the control and fermentation groups. The total phenolic compound content analyzed on the 4th day was 687.92 µg/L in the fermentation group and 633.70 µg/L in the control group.

In conclusion, fermentation with *L. plantarum* for 4 days increased or stabilized the concentration of beneficial phenolic compounds compared to the control group, without negatively affecting the antioxidant capacity. We believe that this study contributes to the introduction of fermented bergamot into daily consumption.

Keywords: *Citrus bergamia*, Bergamot, Phenolic compounds, Antioxidant capacity

Microbial evaluation of fermented beetroot juice produced by probiotic *Lacticaseibacillus paracasei*

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ABSTRACT

Probiotic products have a significant proportion in functional food market, and research on the use of fruits and vegetables instead of dairy products in the production of probiotic products is increasing due to many factors. Red beetroot juice can be produced spontaneously or by probiotic bacteria. It is important to determine a product-specific pasteurization parameter to ensure that the product is microbially safe. Red beetroot is a very valuable plant due to its phenolic components and betalains, which have many important effects on health. The aim of this study is to produce a probiotic drink from red beetroot juice, which is so important for health, and to enable consumers to benefit more from red beetroot. In this study, the red beetroot juice samples produced by probiotic strain *Lacticaseibacillus paracasei* 431®, 17 different runs were created with the Box Behnken experimental design in Responce Surface Methodology. As independent variables; temperature (60-80°C), time (10-30 min.), and fermentation temperature (24-36°C) were selected. To demonstrate the effectiveness of the pasteurization process, total yeast and mold (TYM), and total mesophilic bacteria (TMB) were determined right after pasteurization and before fermentation. The results showed that; before fermentation, TYM and TMB counts of the samples were 0.50-2.87 log CFU/mL and 0.35-4.12 log CFU/mL, respectively. According to the ANOVA test results, models were significant, and also temperature and time were found to be significant for both responses ($p < 0.05$). After fermentation, TYM, TMB and total lactic acid bacteria (LAB) counts of the samples ranged between 8.29-9.12 log CFU/mL, 8.50-9.25 log CFU/mL, and 8.17-9.01 CFU/mL, respectively. Although differences were determined between the microbial loads of the samples at the beginning of the fermentation, the effect of the models determined were found insignificant at the end of the fermentation ($p > 0.05$).

Keywords*:* Beetroot juice, functional food, probiotic vegetable product, *Lacticaseibacillus paracasei, Responce Surface Methodology*

INTRODUCTION

In recent years, consumers' demands for products beneficial for health have been increasing for many reasons. Functional foods, which show the presence of multiple health-beneficial components such as antioxidants, bioactive peptides, vitamins, minerals, prebiotics and probiotics, are of great interest in these products [1]. Probiotic foods are the fastest growing area in functional food production, representing 70% of the functional food market [2]. The global probiotic market is estimated to be worth 61.1 billion US dollars in 2021 and will reach 91.1 billion US dollars by 2026 (Markets and Markets, 2020). It is predicted that the demand for foods containing probiotics will remain high as awareness of the benefits of these products and consumers' desire to purchase premium products combined with probiotics [3], [4]. In addition, the COVID-19 epidemic process, which has been in the whole world recently, has led to a change in the consumption patterns of consumers and this has affected the demand for diet. Fear of being infected and the adoption of healthy lifestyle have also led to increased demand for probiotics [5].

Fruits and vegetables are a good matrix for lactic acid bacteria due to their large amounts of carbohydrates, polyphenols, vitamins, minerals, and dietary fibers [6]. Lactic acid fermentation is an effective biological process that ensures product safety, longer shelf-life, provides probiotic properties, maintains or increases nutritional value, and develops new products which have unique sensory quality [3].

As defined by FAO/WHO (2001), probiotics; are live microorganisms (mainly bacteria and a few yeasts, strains that provide a beneficial health effect on the host) that have positive effects on health when taken into the body in sufficient quantities. We can align many positive effects on health for probiotic bacteria. Among them; a) increasing the nutritional value of food products, b) controlling and lowering serum cholesterol, c) improving the immune system, d) preventing intestinal infections and suppressing antibiotic-associated diarrhea, e) reducing symptoms of lactose intolerance, f) reducing the risk of colon cancer, and g) depending on the type of probiotic strain, there is improvement of gliadin digestion in gluten-containing foods against celiac disease [7]. Because of these functionalities, people tend to consume products containing probiotics. As a result of this increasing trend, marketing efforts aiming to produce new functional food products are emerging. For this purpose, many different food products containing probiotics can be designed and commercialized. Traditionally, fermented milk products have been considered the most excellent carriers for probiotics, but milk-based products may be limited in use due to lactose intolerance, allergies, dyslipidemia, and vegetarianism. In addition, dairy products contain high cholesterol, 75% of the world population suffers from lactose intolerance [8] and for economic reasons for developing countries, they do not contain cholesterol but contain protein, starch, minerals, fiber, vitamins and antioxidants that prevent diseases. fruits, vegetables, cereals and legumes, etc., from products rich in its content. can be good alternatives for designing probiotic products [9], [10].

Red beetroot (*Beta vulgaris L.*) is a flowering plant belonging to the *Amaranthaceae* family. Although its homeland is the Mediterranean Region, it is produced in a wide area, recently extending to America, Europe and India.There are many research on the effects of red beet on human health. In recent years, there has been an increasing interest in the biological activities of red beet, including its positive effects on gastrointestinal health [11]–[13]. Metabolism of the oligo and polysaccharides in red beetroot by the bacteria that make up the gut microbiota has demonstrated the ability of these components to modulate positive gut microbial communities and stimulate the production of specific metabolites indicative of potential prebiotic properties[14]–[17]. For this reason, it is thought that red beet will be a suitable matrix for the development of probiotics and many studies have been carried out on the growth status of different probiotic bacteria in red beetroot juice.

In this study, it was aimed to investigate the effect of different pasteurization treatments and ambient temperature on quality in red beetroot juice production using a probiotic strain *Lacticaseibacillus paracasei* 431.

MATERIAL AND METHODS

Red beetroot and the other ingredients used in the red beetroot production were purchased from a local store in Sivas, Turkey.

Lacticaseibacillus paracasei 431® strain was supplied from CHR Hansen® (Denmark). Strain was kept at -20°C until they use.

Preparation of Red Beetroot Juices

Inoculum was prepared from overnight cultivation of *Lcb. paracasei* 431 at 37°C in a 250-rpm shaking incubator (ISS-3075, Jeio, Tech Lab Companion). At the end of the incubation period, 1 mL of this suspension cultivated in sterile red beetroot juice, and the inoculated under the same incubation conditions. After incubation suspension of *Lcb. paracasei* used in fermentation process.

The beetroot juices were prepared by slicing the fresh vegetables, followed by passing through a juice extractor (Philips, HR1861). The mixture of beetroot juice (62.5%), garlic (1.25%), water (37.5%), salt (2%) and bay leaf was prepared in 300 mL glass flasks, then sealed and subjected to pasteurization. The temperature was controlled with a thermometer in a control flask containing the same amount of components and closed with cotton. After the pasteurization period is completed, the beetroot juices cooled at average 30°C and 2% suspension of *Lcb. paracasei* inoculated into red beetroot juices. The inoculum density was average 5×10^6 CFU/mL. The samples fermented in the temperatures which are given in Table 1 and 2.

Microbiological Analysis

Microbiological analysis of the samples was applied before and after fermentation. With the microbiological analysis, the effect of pasteurization on the microbial quality of the samples just after pasteurization, and also before fermentation was determined. Then, effect of fermentation on microbial quality of the samples was determined after different fermentation temperatures.

Selective media and incubation conditions used for determination of following groups of microorganisms were; counts of total yeast and mold (TYM) on PDA at 25°C for 7 days, counts of mesophilic bacteria (TMB) on PCA at 35°C for 2 days and total number of total number of lactic acid bacteria (LAB) on MRS at 35°C for 3 days. Microbiological quality of juices was investigated using the standard plate method.

Experimental Design

In the study, it was aimed to produce a probiotic drink from red beetroot juice, which is so important for health, and to enable consumers to benefit more from red beetroot**.** The effects of different process parameters on red beetroot juice production were investigated. The influencing factors on determing the parameters can be listed as follows. In conventional fruit and vegetable juice production, the pasteurization temperature and time are applied generally between 60-80°C and 10-30 minutes, respectively [18]–[20]. The intervals were determined in accordance with these studies. Traditional red beet juice is made at room temperature (24–25°C), while LAB bacteria often grow optimally at 30-37°C, hence the temperature range is 24-37°C.

A trial design with the three-factor, and three-level was created as 17 experiments with 5 repetitions at the center point by Box-Behnken experimental design (BBD) in the Response Surface Methodology (RSM) (Table 1). As independent variables; pasteurization temperature (60, 70, 80°C), pasteurization time (10, 20, 30 min.) and fermentation temperature (24, 30, 36°C) were determined. In BBD design, in response TYM, TMB and total count of LAB as microbiological analysis were examined. Fermentation studies were carried out in duplicate.

Table 1: Range and levels of parameters in Box-Behnken experimental design

Parameters	**Levels**		
	-1	0	1
Pasteurization Temperature (°C)	60	70	80
Pasteurization time (min.)	10	20	30
Fermentation temperature (°C)	24	30	36

Statistical analysis

One-way ANOVA test was used to assess the results of the microbial analysis of 17 run obtained through the BBD using MINITAB 20.0 (State College, PA). After fermentation, the effect of 3 independent variables (pasteurization temperature, pasteurization time and fermentation temperature) on the responses was investigated using ANOVA results in BBD at 95% confidence interval. Before fermentation of 17 different run, designed with BBD, samples were pasteurized at the temperatures and times given in the trial design, and after inoculation, the samples were fermented. Therefore, the one-way ANOVA test with a 95% confidence interval was used to examine the impact of just two independent factors (pasteurization temperature and pasteurization time) on the samples before fermentation.

RESULTS AND DISCUSSIONS

The microbiological quality of red beetroot juice before and after fermentation was investigated. For this purpose, counts of TYM and also TMB of the samples were determined after pasteurization (just before inoculation). *Lcb. paracasei* 431 was inoculated into the samples immediately after the pasteurization. TYM, TMB and total LAB counts of the samples were determined end of fermentation.

Microbial quality before fermentation

It was determined that counts of TYM of the samples changed at the levels of 0.50-2.87 log CFU/mL after pasteurization (Table 2). The effect of independent variables was examined by the ANOVA in RSM, and the results are given in Table 3. According to the results of the ANOVA, the pasteurization temperature in linear terms, all square terms and interactions were effective on the counts of TYM ($p<0.05$). Figure 1A showed that highest average count of TYM was obtained at 60℃, and the lowest count was obtained with pasteurization applications at 70℃ and 80℃. A similar study was carried out by Tamme et al. (2010), in their study, after 10 s heat treatment at 65℃, the red beetroot juice samples fermented at 20-22℃. They found average 2.94 log CFU/mL of TYM after pasteurization. In our study, the count of TYM in the samples coded 15 which is pasteurized 60℃ for 10 min., had the highest value among the samples, as 2.87 log CFU/mL (Table 2).

Table 2: Counts of TYM and TMB of the samples before fermentation

Order	Sample code	Pasteurization temperature (℃)	Pasteurization time (min.)	TYM (log CFU/mL)	TMB (log CFU/mL)
1	12	60	20	2.00±1.00	2.29±0.11
2	1	70	10	1.00±0.85	1.74±0.19
3	4	60	30	1.18±2.13	2.09±0.09
4	3	80	30	1.18±0.59	1.06±0.37
5	7	60	20	1.54±1.62	2.97±0.07
6	6	70	20	1.11± 0.01	1.09±0.01
7	14	80	20	1.33±0.33	1.35±0.35
8	2	70	10	1.05±0.35	1.50±0.20
9	13	70	30	1.27±0.57	2.00±0.52
10	5	80	20	1.00±0.00	1.24±0.06
11	15	60	10	2.87±0.22	4.12±0.01
12	8	70	20	1.00±0.01	1.00±1.09
13	11	80	10	1.00±0.01	1.73±0.01
14	9	70	20	0.51± 0.01	1.00±1.09
15	16	70	20	1.45±0.01	0.85±0.15
16	17	70	20	0.50±0.01	0.35±0.01
17	10	70	30	0.85±0.15	1.24±0.06

Table 3: ANOVA summary of counts of TYM of the samples

Source	**DF**	**Adj MS**	**F**	**% contrubition**
Model	5	0.72571	6.15**	77.82
Linear	2	0.72773	6.17**	29.84
Pasteurization temperature (℃) (X_1)	1	1.19359	10.12**	24.23
Pasteurization time (min.) (X_2)	1	0.26187	2.22	5.32
Square	2	0.64821	5.5**	26.36
X_1*X_1	1	1.21276	10.28**	25.27
X_2*X_2	1	0.0518	0.44**	1.05
Interaction	1	0.87669	7.43**	21.62
X_1*X_2	1	0.87669	7.43**	17.80
Residual error	11	0.11792		22.18
Lack-of-Fit	7	0.08867	0.52	8.45
Pure error	4	0.16911		13.73
Total	16			100.00

***p≤0.001, **p≤0.05, *p≤0.01

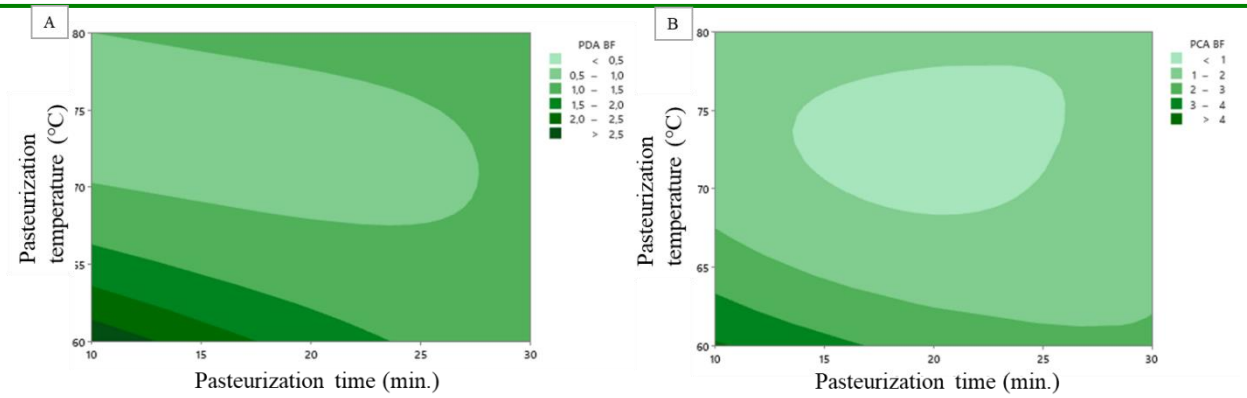

Figure 1 Effect of variables on microbial quality of samples before fermentation, A: counts of TYM, B: counts of TMB numbers

In Table 2, counts of TMB of the red beetroot juice samples were given. It was found that counts of TMB of the samples were at the level of 0.35-4.12 log CFU/mL. The effect of independent variables after pasteurization on the counts of TMB of the samples is given in the ANOVA table (Table 4). It was determined that the linear effects of pasteurization temperature and pasteurization time, as well as all square effects, were significant ($p \leq 0.05$). Interactions were not found to be significant ($p > 0.05$). Figure 1B shows that results were the same as TYM, with the highest average values at 60°C and the lowest values with 70°C and 80°C pasteurization applications. Also, it was observed that the highest number was obtained in 10 minutes, as expected.

Table 4: ANOVA summary of counts of TMB of the samples before fermentation

Source	**DF**	**Adj MS**	**F**	**% contrubition**
Model	5	2.14394	11.1***	77.82
Linear	2	2.76555	14.32***	29.84
Pasteurization temperature (°C) (X_1)	1	4.62731	23.96***	24.23
Pasteurization time (min.) (X_2)	1	0.90379	4.68**	5.32
Square	2	2.36101	12.22**	26.36
X_1*X_1	1	3.26802	16.92**	25.27
X_2*X_2	1	1.21777	6.3**	1.05
Interaction	1	0.46657	2.42	21.62
X_1*X_2	1	0.46657	2.42	17.80
Residual error	11	0.19316		22.18
Lack-of-Fit	7	0.25327	2.88	8.45
Pure error	4	0.08798		13.73
Total	16			100.00

***p≤0.001, **p≤0.05, *p≤0.01

Microbial quality after fermentation

Probiotic *Lc.paracasei* 431 was inoculated into to red beetroot juice samples under aseptic conditions just after pasteurization. Samples fermented at the temperatures which are given Table 5. Approximately 6 log CFU/mL of LAB were found at the beginning of the fermentation process.

At the end of fermentation, counts of TYM of red beetroot juice samples which are fermented with *Lcb. paracasei* 431 were found to be in the range of 8.06-9.01 log CFU/mL, as indicated in Table 5. As can be seen in Table 6, the established model was determined to be insignificant ($P > 0.05$). Although TYM counts of the samples at the beginning of fermentation were determined at the level of 0.50-2.87 log CFU/mL, the effect of pasteurization temperature, all square terms and interaction on the TYM was significant ($P < 0,05$) (Table 3), all effects were found to be insignificant as a result of fermentation ($P > 0.05$) (Table 7).

Table 5: Counts of TYM and TMB of the samples after fermentation

Order	Sample code	Pasteurization temperature (°C)	Pasteurization Time (dak.)	Fermentation temperature (°C)	TYM (log CFU/mL)	TMB (log CFUmL)	LAB (log CFU/mL)
1	12	60	20	36	8.52±0.10	8.50±0.23	8.56±0.10
2	1	70	10	36	8.60±0.10	8.71±0.06	8.58±0.03
3	4	60	30	30	8.06±0.30	9.25±0.30	9.06±0.11
4	3	80	30	30	8.17±0.47	8.28±0.43	8.29±0.48
5	7	60	20	24	8.81±0.01	8.87±0.15	8.79±0.01
6	6	70	20	30	8.60±0.10	8.52±0.80	8.59±0.32
7	14	80	20	24	9.01±0.10	9.26±0.14	8.85±0.07
8	2	70	10	24	8.81±0.04	8.74±0.05	8.63±0.07
9	13	70	30	24	8.75±0.02	8.84±0.05	8.62±0.01
10	5	80	20	36	8.77±0.11	8.60±0.24	8.78±0.19
11	15	60	10	30	8.79±0.10	8.91±0.07	8.85±0.11
12	8	70	20	30	8.86±0.12	8.63±0.23	8.78±0.21
13	11	80	10	30	8.33±0.13	8.54±0.38	8.37±0.16
14	9	70	20	30	8.98±0.10	8.83±0.34	8.78±0.12
15	16	70	20	30	8.37±0.10	9.10±0.15	8.82±0.32
16	17	70	20	30	8.80±0.05	8.80±0.32	9.12±0.16
17	10	70	30	36	8.77±0.10	8.63±0.02	8.45±0.30

Red beetroot juice is also a good growth medium for yeast and moulds due to its nutritional components. The TYM results obtained in the study are similar to other studies. In a study, researchers performed spontaneous fermentation using white cabbage, and stated that at the end of fermentation, TYM in samples could reach up to 6 log CFU/mL with pH values ranging from 4 to 4.3 [21]. Other researchers applied two different combinations of LAB, *Lcb. plantarum* and *Enterococcus faecium* (I.) and *Lpb. pentos* (II) to fresh and wilted whole grain wheat samples and evaluated counts of yeast and mould separately at the end of fermentation. While the pH values of the fresh and wilted whole grain samples of the two combinations were 4 on average, it was determined that the number of moulds that could not be detected at the beginning of the fermentation ranged from 5.3-7.4 CFU g-1DM at the end of the fermentation [22]. [23] determined that while the TYM counts of the samples were at the level of 4.76 log CFU/g before fermentation in the production of spontaneous curly kale drink, it was above 7 log CFU/g on average at the end of the fermentation.

The counts of TMB of the samples was stated to be at the level of 8.28-9.25 log CFU/mL (Table 6). Table 8 shows that the model belongs to counts of TMB of samples was insignificant ($p>0.05$). Just like the TYM, counts of TMB of the samples, which differed from each other at the beginning of the fermentation ($p<0.05$), did not differ significantly at the end of the fermentation ($p>0.05$). Here again, the effective factor is that red beetroot juice is an excellent medium for the growth of mesophilic bacteria. TMB datas obtained in the study are similar to other studies. [23] determined that counts of TMB of the curly kale juice samples were above 7 log CFU/mL on average after 48 hours of fermentation. [24] inoculated 4 different LAB strains (one of them *Lcb. paracasei* LUHS244) into corn samples, which were subjected to various pre-temperature treatments and found that counts TMB ranged between 7.94-8.78 log CFU/ml.

Table 6: Estimated regression coefficients and ANOVA summary TYM of the samples after fermentation

Estimated regression coefficients terms	**ANOVA results**					
	Coefficient	**Source**	**DF**	**Adj MS**	**F**	**% contrubition**
Constant	8.719	Model	9	0.032892	0.39	33.11
Pasteurization temperature (°C) (X_1)	-0.099	Linear	3	0.048272	0.57	16.20
Pasteurization time (min.) (X_2)	0.013	X_1	1	0.079	0.92	8.84
Fermentation temperetaure (°C) (X_3)	-0.09	X_2	1	0.001425	0.02	0.16
X_1*X_1	-0.056	X_3	1	0.064391	0.75	7.20
X_2*X_2	-0.104	Square	3	0.037005	0.43	12.42
X_3*X_3	0.117	X_1*X_1	1	0.013296	0.16	1.46
X_1*X_2	-0.081	X_2*X_2	1	0.045759	0.54	4.55
X_1*X_3	0.012	X_3*X_3	1	0.057327	0.67	6.41
X_2*X_3	0.058	Interaction	3	0.013398	0.16	4.50
S=0.2922711, PRESS=6.29585		X_1*X_2	1	0.026176	0.31	2.93
R^2= 33.11%, R^2(adj)=0%.		X_1*X_3	1	0.000568	0.01	0.06
		X_2*X_3	1	0.013452	0.16	1.50
		Residual error	7	0.085423		66.89
		Lack-of-Fit	3	0.123787	2.19	41.54
		Pure error	44	0.056649		25.35
		Total	16			100.00

***p≤0.001, **p≤0.05, *p≤0.01

As given in Table 6, it was determined that total LAB of the samples after fermentation varied between 8.29 and 9.12 log CFU/ml. As the results of ANOVA, the model built on the total LAB was determined to be insignificant (p>0.05) (Table 9). The effective factor here is that the counts of LAB of all samples are almost the same. Despite the internal and external factors expected from a probiotic beverage, probiotic microorganisms must remain alive in the product until consumed. In the probiotic product, probiotic microorganisms should be at least 6 log CFU/mL and acceptable levels should be 7-8 log CFU/mL All red beet juices produced meet this criteria [25]. Similar results also reported in beverages produced by probiotic *Lcb. paracasei* [1], [26]–[28] and *Lcb. paracasei* [24], [29], [30].

Figure 2 shows the distribution of TYM, TMB and total LAB numbers of the samples before and after fermentation. Although the TYM and TMB values of the samples were determined in a wide range depending on the change in the temperature and time of pasteurization treatments before fermentation, this distribution is limited after fermentation. The effective factor is red beetroot juice is an excellent medium for microorganisms. All samples have good amount of lactic acid in the medium due to fermentation of probiotic *Lcb. paracasei* and also there are other acids in the medium, these cannot prevent the growth of yeast and moulds. The fact that the the higher counts TYM of the samples does not mean that the samples were spoiled. Although the results were not shared here, the samples, whose TYM numbers did not differ from each other at the end of the fermentation, were generally appreciated. We can also say that they are stable in storage (data not shown).

Table 7: Estimated regression coefficients and ANOVA summary TMB of the samples after fermentation

Estimated regression coefficients terms	**ANOVA results**					
	Coefficient	**Source**	**DF**	**Adj MS**	**F**	**% contrubition**
Constant	8.776	Model	9	0.046744	0.47	37.69
Pasteurization temperature (°C) (X_1)	-0.106	Linear	3	0.095873	0.97	25.77
Pasteurization time (min.) (X_2)	0.012	X_1	1	0.089572	0.9	8.03
Fermentation temperetaure (°C) (X_3)	-0.157	X_2	1	0.00113	0.01	0.10
X_1*X_1	0.022	X_3	1	0.196918	0.98	17.64
X_2*X_2	-0.056	Square	3	0.00507	0.05	1.36
X_3*X_3	0.01	X_1*X_1	1	0.002115	0.02	0.15
X_1*X_2	-0.15	X_2*X_2	1	0.013302	0.13	1.17
X_1*X_3	-0.071	X_3*X_3	1	0.000458	0	0.04
X_2*X_3	-0.044	Interaction	3	0.03929	0.4	10.56
S=0.315183, PRESS=8.31		X_1*X_2	1	0.090133	0.91	8.08
R^2= 37.69%, R^2(adj)=0%.		X_1*X_3	1	0.020095	0.2	1.80
		X_2*X_3	1	0.007641	0.08	0.68
		Residual error	7	0.099341		62.31
		Lack-of-Fit	3	0.166704	0.41	44.81
		Pure error	4	0.048818		17.50
		Total				100.0

Table 8: Estimated regression coefficients and ANOVA summary LAB of the samples after fermentation

Estimated regression coefficients terms	**Coefficient**	**ANOVA results**				
		Source	**DF**	**Adj MS**	**F**	**% contrubition**
Constant	8.816	Model	9	0.037113	0.58	42.54
Pasteurization temperature (°C) (X_1)	-0.1203	Linear	3	0.049392	0.77	18.87
Pasteurization time (min.) (X_2)	-0.0032	X_1	1	0.115777	1.8	14.74
Fermentation temperetaure (°C) (X_3)	-0.0636	X_2	1	0.00008	0	0.01
X_1*X_1	-0.001	X_3	1	0.032318	0.5	4.12
X_2*X_2	-0.173	Square	3	0.051551	0.8	19.70
X_3*X_3	-0.073	X_1*X_1	1	0.000002	0	0.11
X_1*X_2	-0.073	X_2*X_2	1	0.125582	1.95	16.76
X_1*X_3	0.039	X_3*X_3	1	0.02221	0.34	2.83
X_2*X_3	-0.03	Interaction	3	0.010396	0.16	3.97
S=0.253879, PRESS=8.31, R^2= 42.54%, R^2(pred)=0%,		X_1*X_2	1	0.021358	0.33	2.72
		X_1*X_3	1	0.006154	0.1	0.78
		X_2*X_3	1	0.003676	0.06	0.47
		Residual error	7	0.064455		57.46
		Lack-of-Fit	3	0.100467	2.68	38.39
		Pure error	44	0.037445	0.58	19.08
		Total				100

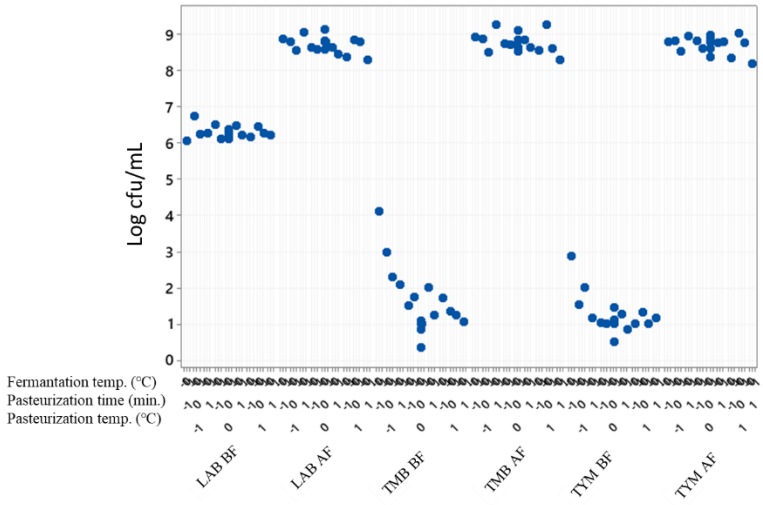

Figure 2. Change of microbial load of samples before and after fermentation, *LAB BF: Lactic acid bacteria before fermentation, LAB AF: Lactic acid bacteria after fermentation, TMB BF: Total mesophilic bacteria before fermentation, TMB AF: total mesophilic aerobic bacteria after fermentation; TYM BF: total yeast and moulds before fermentation.

CONCLUSION

As a result of the study, it has been proven that vegetable juices such as red beet juice are a very good environment for the development of *Lcb. paracasei* 431. Additionally, when a fermentation is performed in red beetroot juice, at the beginning of the fermentation TYM and TMB are below 2.87 log CFU/mL and 4.12 log CFU/mL, respectively, the difference between the final TYM and TMB values of the samples is insignificant regardless of fermentation temperature. Red beetroot juice is also a good growth medium for yeast and moulds, mesophilic bacteria and LAB due to its nutritional components.

It is understood from the results that the lactic acid developing in the medium due to fermentation and other acids in the medium do not prevent the growth of yeast and moulds. Lactic acid bacteria, yeast and molds

and aerobic mesophilic bacteria grew together during fermentation period of samples, contributing to the characteristics of the final product, presumably by producing organic acids, carbon dioxide and other unpredictable flavour compounds [31]. The concurrence and free proliferation of lactic acid bacteria and yeasts, as was observed in this study, is a common circumstance in fermentation of food and beverages.

Therefore, there is no need to make extreme applications when choosing the pasteurization temperature and duration in vegetable juice producing. Choosing softer treatments will be more beneficial in terms of the chemical components of the product. Microbial analysis alone will not be sufficient when choosing spontaneous or controlled fermentation in vegetable juice production. The effects of these production techniques on chemical components should also be investigated. These analysis results should also be taken into account in the selection of the production method in fruit/vegetable juice making. Since red beetroot contains components that have many positive properties on health, the change of these components should not be ignored in the production of a probiotic beverage.

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Bioactive Properties of *Pistacia Lentiscus* Leaves and Fruits

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ABSTRACT

Pistacia lentiscus is a plant species belonging to the Anacardiaceae family, characterized by evergreen leaves. This plant, which is widely distributed in the Mediterranean region with two subspecies: naturally growing *Pistacia lentiscus* L. in shrub form and the cultivated *Pistacia lentiscus* var. Chia. In this review, it is aimed to provide information about the phenolic compounds examined in *P. lentiscus* leaf and fruit extracts and their bioactive properties such as antioxidant and antimicrobial activity. Phenolic acids, flavonoids and their derivatives and anthocyanins are the main phenolic groups found in *P. lentiscus* leaves and fruits. Due to the high phenolic content of leaf and fruit extracts, they exhibit bioactive potential such as antioxidant and antimicrobial. Phenolic compounds produced by *P. lentiscus* using different biosynthesis pathways may differ quantitatively and qualitatively depending on various factors of the plant. However, its bioactive properties may vary depending on its phenolic composition. These factors affect the phenolic content and bioactive properties of the plant. They include the genetic origin of the plant, extraction method, gender, and growth period of the plant, geographical origin and location, climatic conditions, soil fertility, and harvest time, among others.

Keywords: *Pistacia lentiscus*, leaves, fruits, phenolic, bioactive properties

INTRODUCTION

Plants naturally produce bioactive compounds with protective properties. These bioactive compounds found in plants are primarily composed of phenolic compounds (Drioiche et al., 2023). Phenolic compounds are secondary metabolites that contain one or more hydroxyl groups combined with an aromatic ring in their structures (Remila et al., 2015; Dragović et al., 2020). These compounds, categorized into different subclasses such as flavonoids, tannins, phenolic acids, and lignans, play a crucial role in the prevention and/or treatment of various diseases due to their diverse bioactive properties, including antioxidant and antimicrobial activities (Drioiche et al., 2023). Produced in living cells to protect cells from damages caused by free radical chain reactions, these compounds also have the ability to block these reactions. Additionally, they can function as enzyme inhibitors or metal chelating agents, potentially safeguarding cells from potential harmful effects (Djebari et al., 2021).

Pistacia lentiscus, which belongs to the Pistacia genus and is in the Anacardiaceae family, is an evergreen shrub plant with evergreen leaves. It is generally known as the mastic tree because a resin called gum is secreted from its trunk (Gardeli et al., 2008). Today, there are two subspecies: *Pistacia lentiscus* L., which grows naturally in shrub form, and *Pistacia lentiscus* var. Chia, which is cultivated (Daferera et al., 2002). It is generally found in maquis in the coastal areas of the Mediterranean region in countries such as Greece, Turkey, Tunisia, Morocco, Spain, Algeria, and Italy (Trabelsi et al., 2012). *P. lentiscus* is a dioecious plant (Milia et al., 2021), and the female trees of this plant have small fruits containing a single seed, which are initially green and change from dark red to black as they mature (Drioiche et al., 2023). Due to the gender difference of the mastic tree, there are differences in the leaves of the male and female trees and their leaves are double-pieced, oblong or oblong-lanceolate (Kılınç, 2013).

Various studies have indicated that various parts of the *P. lentiscus* plant, such as leaves, fruits, and twigs, are rich in phenolic compounds (Rodríguez-Pérez et al., 2013; Pacifico et al., 2014; Dragović et al., 2020; Al-Zaben et al., 2023). In these studies, it was stated that the main phenolic groups found in the plant are phenolic acids, flavonoids, flavonols and anthocyanins. Studies conducted on different parts of the plant have shown that the total phenol content is higher in leaves compared to fruits and other parts (Yemmen et al., 2017; Yosr et al., 2018).

Studies have reported that *P. lentiscus* leaves and fruits possess a rich phenolic content and, consequently, exhibit high bioactive properties. In this review, it is aimed to summarize the phenolic compounds examined in the leaf and fruit extracts of the *P. lentiscus* plant, the bioactive properties such as antioxidant and antimicrobial activity displayed by these compounds, and the various factors affecting them.

Phenolic compounds of *Pistacia lentiscus*

In the *P. lentiscus*, the primary components are phenolic acids and flavonoids (Dragović et al., 2020; Detti et al., 2020). Instrumental devices such as liquid chromatography (LC), high-performance liquid chromatography (HPLC), high-performance liquid chromatography with diode array detector and mass spectrometry (HPLC-DAD-MS-ESI) are utilized for the separation, identification, and quantification of phenolic compounds in *P. lentiscus*, which is rich in phenolic components (Sehaki et al., 2023). These techniques allow obtaining qualitative and quantitative information about the phenolic profile of *P. lentiscus*. In a study on this subject, using ethyl acetate and methanol fractions of *P. lentiscus* leaves, it was reported that a high polyphenol content was detected in the extracts, representing 7.5% of the dry weight of the leaf. For the separation, identification, and quantification of these polyphenols, methods such as HPLC, HPLC-DAD, HPLC-MS, and 1H- and ^{13}C-NMR (Carbon-13 nuclear magnetic resonance) were employed. It was indicated that three main classes of secondary metabolites were identified: (i) gallic acid and galloyl derivatives of both glucose and quinic acid; (ii) flavonol glycosides such as myricetin and quercetin glycosides; and (iii) anthocyanins such as delphinidin 3-*O*-glucoside and cyanidin 3-*O*-glucoside. All of these compounds have been reported to be potent antioxidant polyphenols with effects in the prevention of chronic and inflammatory diseases (Romani et al., 2002).

Studies have reported the significant presence of phenolic compounds such as phenolic acids, flavonoids, and anthocyanins in *P. lentiscus* fruits (Pachi et al., 2021; Tebbi et al., 2023). The chemical structures of some of the major phenolic compounds found in *P. lentiscus* are shown in Figure 1. In a study on this subject, the antioxidant properties of fruit phenolic extracts were associated with dominant phenolics, namely gallic acid, digalloyl quinic acid derivatives, and quercetin (Yemmen et al., 2017). Some studies have reported the presence of gallic acid, galliol derivatives, and flavanol glycosides in *P. lentiscus* leaves (Rodríguez-Pérez et al., 2003; Pacifico et al., 2014; Al-Zaben et al., 2023). The black ripe fruits of *P. lentiscus* exhibit high antioxidant activity due to their richness in anthocyanins, thus also possessing significant antimicrobial activity (Rodríguez-Pérez et al., 2013; Mezni et al., 2015). In addition, these fruits' oils also have antioxidant, antifungal, antimicrobial, antiulcer and anticancer properties (Akdemir et al., 2015). In a previous study, the anthocyanin contents of *P. lentiscus* fruits were determined by HPLC-DAD-MS. Accordingly, the major anthocyanin of *P. lentiscus* fruit is defined as cyanidin 3-*O*-glucoside; it was also reported that delphinidin 3-*O*-glucoside and cyanidin 3-*O*-arabinoside were found in small amounts (Longo et al., 2007).

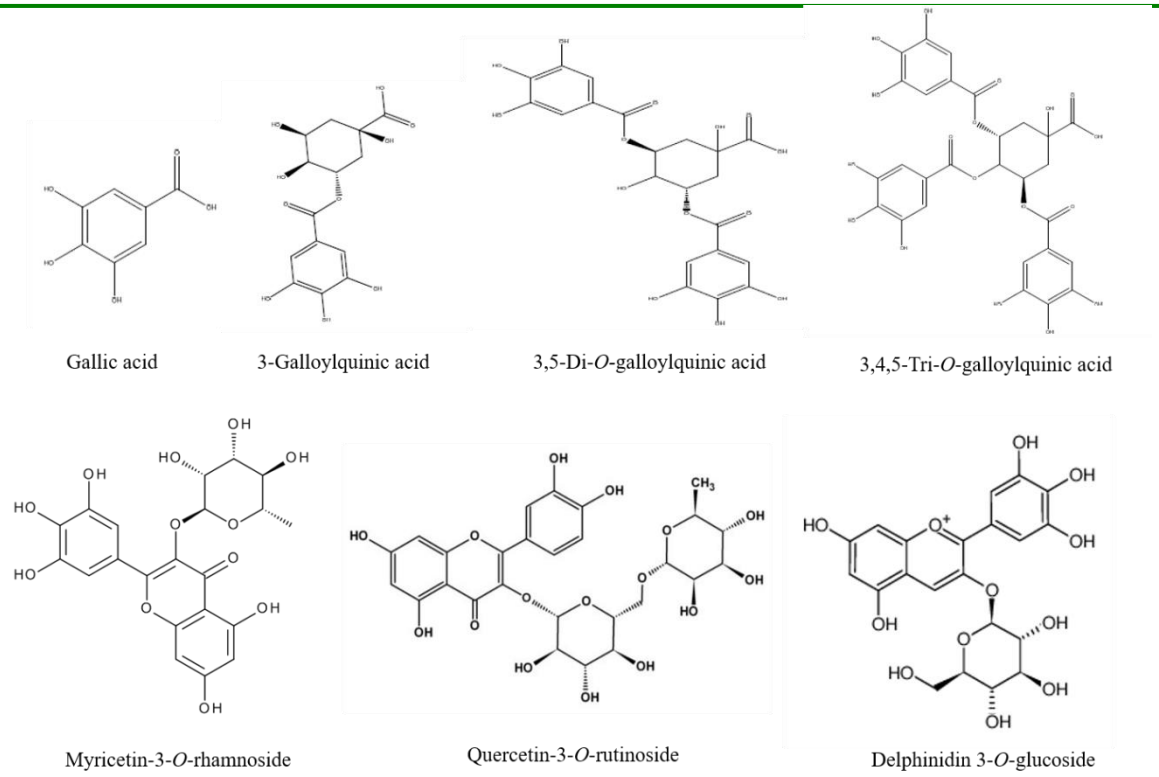

Figure 6. The chemical structures of some of the major phenolic compounds in *P. lentiscus*

The leaves of *P. lentiscus*, like its fruits, are rich in phenolic acids and flavonoids. In a study conducted on this subject, UPLC-MS analysis revealed the presence of a total of 11 polyphenolic compounds in *P. lentiscus* leaves. Among these, six were identified as flavonol glycosides (myricetin-rutinoside, myricetin-glucoside, quercetin-rutinoside, myricetin-rhamnoside, quercetin-glucoside, quercetin-rhamnoside), and five were phenolic acids (glucogallin, gallic acid, galloyl quinic acid, digalloyl quinic acid, trigalloyl quinic acid) (Remila et al., 2015). El Bishbishy et al. (2020) conducted a study examining the phenolic compounds of *P. lentiscus* L. leaves using UHPLC-ESI-MS. They identified flavonoid glycosides, catechins, various phenolic acids, and their derivatives in the leaves, with 3,5-*O*-digalloylquinic acid identified as the most dominant compound. Another study investigating the phenolic compounds of *P. lentiscus* L. leaves using UHPLC-QTOF/MS identified a total of 18 compounds. These compounds were categorized into four main groups: cyclic compounds, galloyl derivatives, flavonoids (flavonols and flavones), and monoterpenoid glycosides (Stefi et al., 2021). In a study by Zahouani et al. (2021) examining the phenolic compounds of *P. lentiscus* leaves from Tunisia using HPLC-MS, they detected a total of seven compounds, including 1,5- or 3,5-digalloylquinic acid, ellagic acid, catechin, luteolin-7-glucoside, isoquercetin or hyperoside, kaempferol rutinoside, and rutin. Among these, isoquercetin or hyperoside and luteolin-7-glucoside were the most abundant flavonoids, while catechin and ellagic acid were identified as the most abundant flavonol and phenolic acid, respectively.

Factors Influencing the Phenolic Compounds

Phenolic compounds found in the leaves and fruits of the *P. lentiscus* can be produced in different amounts and types. These compounds are synthesized through different biosynthetic pathways in the plant, leading to quantitative and qualitative variations based on the plant's developmental stages. However, it may also vary depending on the genetic origin of the plant and environmental factors (Verma and Shukla, 2015; Dragović et al., 2020). Studies on this have reported that phenolic compounds are influenced by various factors, including extraction method, plant gender and growth stage, geographical origin and location, climate conditions, soil fertility, and harvest time, among others (Romani et al., 2002; Dragović et al., 2020). In a study conducted in Spain, it was reported that the total amount of terpenes in the leaves of *P. lentiscus* showed a positive correlation with soil moisture but a negative correlation with air temperature (Llusia et al., 2006). In another study, it was stated that there were significant differences in the phenolic contents of *P. lentiscus* leaves collected at different phenological stages and that the synthesis of phenolic compounds was significantly related to plant phenological stages (Dragović et al., 2020).

There may be quantitative and qualitative differences in phenolic compounds depending on the extraction method and solvent used to extract the phenolic compounds of the leaves and fruits of the *P. lentiscus*. In a study by Dahmoune et al. (2014), optimized the microwave-assisted extraction method to examine the phenolic compounds of *P. lentiscus* L. leaves and compared the extraction conditions containing the highest total phenolic substances with ultrasound-assisted and traditional extraction methods. The total phenolic content of leaf samples was determined as 185.69, 142.76, and 178.00 mg GAE/g dry weight for microwave, ultrasound, and traditional extraction methods, respectively, and reported that the highest value was reached in the microwave-assisted extraction method. Elez Garofulić et al. (2020) extracted the phenolic of *P. lentiscus* L. leaves and fruits with different methods (microwave-assisted and conventional method) and examined their polyphenolic profiles with UPLC/ESI-MS^2. It has been reported that microwave-assisted extraction is an effective method for the separation of *P. lentiscus* L. leaves and fruit polyphenols. It was stated that the total phenolic content in leaves and fruits obtained with the optimized microwave-assisted extraction was similar to that obtained with conventional extraction, but microwave-assisted extraction was carried out in a shorter time. According to the analysis results, it was detected 33 compounds in the leaves and 34 compounds in the fruit and reported that the most dominant phenolic compound group in both leaves and fruit was flavonols. The most abundant compounds in both leaves and fruits were reported to be myricetin rhamnoside and myricetin glucuronide, respectively.

Phenolic compounds found in *P. lentiscus* vary depending on the geographical origin, variety, gender, phenological stages, harvest time and environmental conditions of the plant. Dragović et al. (2020) obtained *P. lentiscus* leaves from four different regions of the Adriatic coast and in three different phenological stages (early flowering, early fruiting and late fruiting) and they evaluated the effects of these factors on the phenolic content of the leaves using two different extraction solvents (80% methanol, 80% ethanol). According to the findings, they reported that different regions, phenological stage and extraction solution had a significant effect on the diversity of phenolic compounds. They reported that the early flowering period is the most suitable period for maximum total phenolic content, and the most suitable period for total flavonol glycoside content is the early fruiting period. However, researchers also stated that methanol solution is more efficient than ethanol. In total, seven phenolic acids and five flavonol glycosides were identified in the samples, and 5-*O*-galloyl-quinic acid in phenolic acids and myrcetin-3-*O*-rhamnoside in flavonol glycosides were identified as the most dominant compounds. Aissat et al. (2022) investigated the anthocyanins and phenolic compounds of *P. lentiscus* L. fruit obtained from Algeria in five different physiological stages. They reported that in this study, a total of 30 compounds were detected, including nine anthocyanins, seven flavanols, seven flavonols, two phenolic acids, two flavanones, a stilbene, a flavanonol and a dihydrochalcone. It has been stated that flavonols were the main compounds of raw fruits, while anthocyanins were the main compounds of ripe fruits. They also reported that *(E)*-piceid and protocatechuic acid were identified for the first time in *P. lentiscus* L. fruits.

Bioactive properties of *Pistacia lentiscus*

Research on the *P. lentiscus* L. plant indicates a broad range of potential uses for various parts of the plant, such as leaves and fruits, in the treatment of various diseases. Polyphenols, including phenolic acids and flavonoids found in the plant have significant potential for health benefits (Djebari et al., 2023). Various bioactive properties such as antioxidant, anti-diabetic, anti-tumor, and anti-bacterial effects of different parts of the plant like leaves and fruits have been stated in studies (Marone et al., 2001; Botsaris et al., 2015; Remila et al., 2015; Mehenni et al., 2016; Boucheffa et al., 2021). The effectiveness of bioactive properties varies depending on the richness of polyphenols and, in particular, the concentrations of tannins, flavonoids and phenolic acids in the polyphenol mixture (Milia et al., 2021). Furthermore, the high polyphenol content in the leaves and fruits of the *P. lentiscus*, and thus its bioactive properties, have been utilized in traditional medicine since Ancient Greek times for the treatment of various diseases (Trabelsi et al., 2012; Remila et al., 2015; Aissi et al., 2016). It has traditionally been used to treat conditions such as hypertension, cough, sore throat, eczema, abdominal pain, toothache, kidney disorders, jaundice, skin problems and diarrheal diabetes (Nabila et al., 2008; Saiah et al., 2016; Bouyahya et al., 2016). al., 2021). Today, *P. lentiscus* is used in the treatment of various diseases such as hypertension, heart, gastrointestinal, cough, sore throat and eczema in many countries including Iran, Tunisia, Morocco and Italy (Milia et al., 2021). Numerous studies have underlined that the phenolic compounds found in the structure of *P. lentiscus* are important in human health and especially in diseases related to oxidative stress (Manach et al., 2004; Arranz et al., 2012). Oxidative stress occurs when free radical molecules produced in the body damage cells and tissues, and this can cause cellular damage and various diseases as a result of a series of chain reactions (Drioiche et al., 2023).

Additionally, some studies suggest that these compounds play a crucial role in delaying the development of cancer, diabetes, cardiovascular, and neurodegenerative diseases (Sidor and Gramza-Michałowska, 2015; Sehaki et al., 2023). The leaves and fruits of the *P. lentiscus* also have medicinal properties reported in many traditional pharmacopoeias. The oil obtained from the fruit of this plant is widely used as a traditional therapeutic in Eastern Algeria and is often applied locally to treat burns, and it has been stated that it has an analgesic effect (Pachi et al., 2021; Tebbi et al., 2023; Djebari et al., 2023). Additionally, this oil is used in the treatment of various diseases such as diabetes, stomach diseases (such as ulcers), asthma (Siano et al., 2020). The essential oils from *P. lentiscus* leaves are also noted for significant antimicrobial and antibacterial activity (Mecherara-Idjeri et al., 2008).

To evaluate the antioxidant capacity of *P. lentiscus*, which is one of its bioactive properties, ferric-reducing power (FRAP), oxygen radical absorbance capacity (ORAC), free radical scavenging (DPPH - 2,2-diphenyl1-picrylhydrazyl), ABTS (2,20-azino- bis-(3ethylbenzthiazoline-6-sulfonic acid), total antioxidant capacity (phosphomolybdenum, TAC) is used in various methods. Some of the studies examining the antioxidant activities of *P. lentiscus* leaves and fruits are given in the Table 1. The table includes the origin of the plant, extraction type, antioxidant activity method used and the result. In a study on this subject, they examined the antioxidant activities of *P. lentiscus* L. leaves using different extraction solutions (butanol, acetone, methanol and water). The antioxidant activity values of the leaves ranged from 123.9 to 510.3 mg Trolox/g extract using the DPPH method and from 92.4 to 384.6 mg Trolox/g extract using the ABTS method. The study indicated that the highest antioxidant activity values were achieved with methanol extraction in both methods (Botsaris et al., 2015). In another study involving *P. lentiscus* L. leaves obtained from Morocco, different solvents (hexane, ethyl acetate, ethanol, and water) were used for extraction. In this research, where they examined the total phenolic content and antioxidant activities of the extracts, they found that the extract with the lowest phenolic content was obtained with hexane (30.94 mg GAE/g), while the highest was with water (125.02 mg GAE/g). They reported that aqueous and ethanolic extracts, having higher total phenolic contents, consequently exhibited greater antioxidant activity (Labhar et al., 2023).

P. lentiscus also exhibits antimicrobial properties due to its phenolic compounds. Some of the studies examining the antimicrobial activities of *P. lentiscus* leaves and fruits are given in the Table 2. The table includes the origin of the plant, the type of extraction, the type of microorganism used and their activity against these microorganisms (positive: -+, negative:-). In a study investigating the antimicrobial activity, *P. lentiscus* was evaluated against *Staphylococcus aureus* (gram positive) and *Esherichia coli* (gram negative) using the agar disc diffusion method and minimal inhibitory concentration methods. Four different extraction methods (percolation, infusion, boiling, and methanol extraction) were evaluated. According to the results, the antibacterial activity of *P. lentiscus* against the tested bacteria showed that gram-negative strains were more resistant than gram-positive ones; it was stated that the highest inhibitory activity diameter (25.5 mm) was obtained by the boiling method (Missoun et al., 2017). Djebari et al. (2021) subjected the leaves and fruits of the *P. lentiscus* L. plant to the process of maceration, and the antibacterial activity of these products against 9 bacterial strains (*Staphylococcus aureus, Staphylococcus epidermidis, Bacillus subtilis, Listeria innocua, Escherichia coli, Pseudomonas aeruginosa, Acenitobacter baumannii* and *Salmonella* sp (hospital strain)) was evaluated according to the disk diffusion method. It was reported that both samples had an antimicrobial effect against the tested strains and the largest inhibition zone was created against *B. subtilis*. In another study on *P. lentiscus* fruits, it was reported that both ripe and raw fruits showed antimicrobial effects against *E. coli* and *S. aureus* according to the disk diffusion method, and the highest inhibition zone diameter was in raw fruits (Ayad et al., 2023).

Factors affecting bioactive properties

The methods used in the extraction of phenolic compounds, along with factors such as the region where the plant is cultivated, climate and soil conditions, gender, variety, and harvest time, also influence the bioactive properties (Dragović et al., 2020). In a previous study, the total phenolic contents and antimicrobial activities of aqueous and methanolic extracts of *P. lentiscus* leaves obtained from Saudi Arabia were examined. Accordingly, it was reported that the methanolic extract (28.4 mg GAE/g) had a higher phenolic content than the aqueous extract. Similarly, it has been reported that methanolic extract has more antimicrobial activity than aqueous extract. Consequently, the maximum inhibition zone diameters for nine different bacterial species sensitive to methanolic extracts were found to be in the range of 7-11 mm (Al-Zaben et al., 2023). In another study, extracts of three different parts of *P. lentiscus* L. (leaves, branches, and fruits) were prepared using solvents of varying polarity (hexane, dichloromethane, ethyl acetate, and methanol), and their antiviral activities against herpes simplex virus type 2 (HSV-2), coxsackievirus type B3, and adenovirus type 5 were examined.

According to this study, only the methanol extraction of the branch sample showed significant antiviral activity against HSV-2 (Bouslama et al., 2020).

There are studies that harvest time affects the biological activity of the *P. lentiscus*. Mezni et al. (2014) aimed to optimize the antioxidant activity of *P. lentiscus* L. fruit by determining the optimal harvest period. For this purpose, fruit samples were harvested at three different maturity stages: raw, ripe and over-ripe, and the oils of these fruits were extracted by the Soxhlet extraction method. According to the study results, the lowest antioxidant activity value was detected in ripe fruit oil. Yosr et al. (2018) evaluated the total phenolic, flavonoid and condensed tannin contents of different parts (leaf, flower, ripe and immature fruit and branch) of *P. lentiscus* females and males during four harvest periods (vegetative dormancy, full flowering, early and late fruit formation). It was reported that significant quantitative and qualitative differences were observed in phenolic composition depending on gender, plant part and collection periods. The highest total phenolic, flavonoid, and condensed tannin contents were found in the leaves and flowers of male trees, and these significantly decreased during the vegetative maturation period. It was found that the antioxidant activity of extracts obtained with acetone varied significantly among plant parts. They stated that the lowest averages of IC_{50} were observed in leaves in the vegetative and flowering stages, respectively. Selim et al. (2022) showed the antimicrobial activities of the methanol extract of *P. lentiscus* L. barks against 13 different microorganisms (*Bacillus cereus, Salmonella paratyphi, Enterococcus feacalis, Saccharomyces cerevisiae, Serratia marcescens, Staphylococcus aureus, Acinetobacter baumannii, Aeromonas hydrophila, Brevundimonas vesicularis, Klebsiella pneumoniae, Escherichia coli, Candida albicans,* and *Pseudomonas aeruginosa).* It was reported that the extracts showed antimicrobial activity against the examined microorganisms and had an inhibition zone varying between 10-25 mm. In a recent study Anagnostou et al. (2023), the antimicrobial properties of three different extracts (ethyl acetate, methanol and water) of leaves of the *P. lentiscus* var. Chia species were evaluated. It was stated that methanol and water extracts showed significant selective activity against pathogenic Mucorales but did not exhibit activity against Aspergilli (*Aspergillus nidulans, Aspergillus fumigatus*) and bacteria (*Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis*).

Table 8. Antioxidant activities of *P. lentiscus* leaves and fruits reported in some previous study

Plant origin	Plant material	Extract type	Method type	Antioxidant activity	References
Greece	Aerial parts	Methanolic extract	FRAP	84.6–131.4 mmol Fe^{2+}/l	Gardeli et al., 2008
Italy	Leaves	Methanolic extract	DPPH FRAP	2.9 µg/ml extract 0.6 µg/ml extract)	Pacifico et al., 2014
Algeria	Leaves	Conventional extraction Ultrasound-assisted extraction accelerated solvent extraction	ORAC	671.07 µmol Trolox/g 517.52 µmol Trolox/g 257.07 µmol Trolox/g	Dahmoune et al., 2014
Algeria	Leaves	Ethanolic extract	ORAC	5865 µmol Trolox Equivalent/100 g	Remila et al., 2015
Algeria	Fruits	Ethanolic extract	ORAC	3664 µmol Trolox/100g	Remila et al., 2015
Cyprus	Fruits	butanol, acetone, methanol and water extract	DPPH	70.2-387.6 mg Trolox/g	Botsaris et al., 2015
Morocco	Leaves	Methanolic extract	DPPH FRAP	IC_{50}: 17.22 µg/ml 309.60 mg Ascorbic acid Equivalent/g	Salhi et al., 2019
Croatia	Leaves	Microwave-Assisted Extraction	ORAC	538.41 µmol Trolox/g	Elez Garofulić et al., 2020
Croatia	Fruits	Microwave-Assisted Extraction	ORAC	386.82 µmol Trolox/g	Elez Garofulić et al., 2020
Morocco	Leaves	Aqueous extract Methanolic extract Ethanolic extract	TAC	488.16 mg Ascorbic acid Equivalent/g 239.89 mg Ascorbic acid Equivalent/g 352.76 mg Ascorbic acid Equivalent/g	Barbouchi et al., 2020
Morocco	Fruits	Aqueous extract Methanolic extract Ethanolic extract	TAC	298.53 mg Ascorbic acid Equivalent/g 207.91 mg Ascorbic acid Equivalent/g 217.91 mg Ascorbic acid Equivalent/g	Barbouchi et al., 2020
Algeria	Fruits	Methanolic extract	FRAP TAC	2970.39 mg GAE/100g 111.11 mg GAE/g	Ayad et al., 2023

Table 9. Antimicrobial activities of *P. lentiscus* leaves and fruits reported in some previous studies

Plant origin	Plant material	Extract type	Microorganism	Antimicrobial activity	References
Algeria	Leaves and steam	Methanol extract	*Staphylococcus aureus* *Escherichia coli*	+ -	Missoun et al., 2017
Italy	Fruit	Methanol extract	*Staphylococcus aureus* *Staphylococcus epidermidis* *Escherichia coli* *Klebsiella pneumoniae*	+ - - -	Mandrone et al., 2019
Italy	Leaves	Methanol extract	*Staphylococcus aureus* *Staphylococcus epidermidis* *Escherichia coli* *Klebsiella pneumoniae*	+ + - +	Mandrone et al., 2019
Algeria	Leaves	Ethanol extract	*Staphylococcus aureus* *Listeria innocua* *Escherichia coli* *Klebsiella pneumoniae* *Pseudomonas aeruginosa* *Citrobacter freundii*	+ + + - + -	Bakli et al., 2020
Algeria	Leaves	Methanol extract	*Staphylococcus aureus* *Bacillus subtilis* *Listeria innocua* *Pseudomonas aeruginosa* *Escherichia coli*	+ + + + +	Djebari et al., 2021
Algeria	Fruits	Methanol extract	*Staphylococcus aureus* *Bacillus subtilis* *Listeria innocua* *Pseudomonas aeruginosa* *Escherichia coli*	+ + + + +	Djebari et al., 2021
Libya	Leaves	Aqueous extract	*Staphylococcus aureus* *Pseudomonas aeruginosa* *Proteus mirabilis*	+ - +	Alhadad et al., 2022

CONCLUSION

The diverse parts of *P. lentiscus*, such as leaves and fruits, have been identified as rich sources of phenolic content in various studies. The main phenolic compounds of the plant consist of phenolic acids such as gallic acid and galloyl derivatives and flavonols like quercetin and myricetin glycosides. In addition, it contains flavanols such as epicatechin, catechin, epi(gallo)catechin gallate. Due to its rich phenolic profile, *P. lentiscus* also exhibits bioactive properties including antioxidant and antimicrobial effects. It has also been determined that leaves, in general, have more phenolic content than fruits. Extracts obtained from different parts of the *P. lentiscus* plant like leaves and fruits can be used as natural antioxidant sources. However, further pharmacological investigations of these polyphenols are required through clinical studies to determine their usage and limits in humans. This could potentially lead to the development of a natural product that serves as an alternative to artificial antioxidants in the food, pharmaceutical, and cosmetic industries.

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Assessing antimicrobial resistance in *E. coli* isolated from salad vegetables in uae: phenotypic and genomic characterization

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ABSTRACT

The present study investigated the counts, antimicrobial resistance profile, and genome-based characterization of *Escherichia coli* in 11 different types of fresh salad vegetable products (n = 400) sampled from retailers in the United Arab Emirates. *E. coli* was detected in 30% of the tested items, with 26.5% of the samples having an unsatisfactory level (≥ 100 CFU/g) of *E. coli*, notably arugula and spinach. The study also assessed the effect of the variability in sample conditions on *E. coli* counts and found, based on negative binominal regression analysis, that samples from local produce had a significantly higher (P-value < 0.001) *E. coli* count than imported samples. The analysis also indicated that fresh salad vegetables from the soil-less farming system (e.g., hydroponic) had significantly (P-value < 0.001) fewer *E. coli* than those from conventional produce. The study also examined the antimicrobial resistance in *E. coli* (n = 145) recovered from fresh salad vegetables and found that isolates exhibited the highest resistance toward ampicillin (20.68%), tetracycline (20%), and trimethoprim-sulfamethoxazole (10.35%). A total of 13.79% (20/145) of the *E. coli* isolates exhibited a multidrug-resistant phenotype, all from locally sourced leafy salad vegetables. The study further characterized 18 of the 20 multidrug-resistant *E. coli* isolates using whole-genome sequencing and found that the isolates had varying numbers of virulence-related genes, ranging from 8 to 25 per isolate. The frequently observed genes likely involved in extra-intestinal infection were CsgA, FimH, iss, and afaA. The β-lactamases gene blaCTX-M-15 was prevalent in 50% (9/18) of the characterized *E. coli* isolates. The study highlights the potential risk of the spread of antimicrobial resistance bacteria and resistance genes associated with consuming leafy salad vegetables and emphasizes the importance of proper food safety practices, including appropriate storage and handling of fresh produce.

Investigations of thermal treatment and extraction process on the micro- plastic profile in shrimp

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ABSTRACT

Microplastics (MPs) are considered an emerging pollutant that directly bio -accumulated from water bodies to industrial seafood products. Due to the high MPs contamination exposure in water bodies, aquatic animals are more perishable from MPs accumulation. Ingestion of MPs in crustacean species is known at a high level. In this study, the thermal processing and extraction time on the characterization of MPs in the shrimp. MPs bioaccumulation was determined in both raw and boiled deep water pink shrimps by acid-based extraction method. Two different extraction times were applied to both raw and boiled shrimp to determine the potential impact of the extraction process on the detection of MPs in shellfish. Following different extraction applications, MPs were detected in all the samples, accounted by a stereo microscope morphologically and characterized by Raman spectroscopy, and visualized by Scanning Electron Microscope. The results reveal that the accumulation of MPs was reduced at the 15% level by boiling.

Keywords: micro plastic, seafood, thermal process, food safety, extraction method

Impact of oat-drink residue flour on the white bread's dough properties and baking characteristics

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ABSTRACT

The aim of this work is to study the structure-function relationship of dry fractionated oat drink residue (DFOR) as a technological ingredient, using bread as a model system. Dried oat drink residue was mechanically fractionated into three different particle sizes DFOR1:<150µm, DFOR2:150-224µm, and DFOR3:224<300µm and blended with bread wheat flour at 10% and 20% substitution levels for bread making. The blended flours and bread samples were assessed for their dough mixing and bread technological characteristics. Results from Mixolab, Rapid Visco Analyser, and gel strength showed that, inclusion of DFOR exhibited a higher ($p<0.05$) water absorption values compared with control. Similarly, both final viscosity and setback values were significantly increased ($p<0.05$) particularly at 10% substitution levels, whereas 10% mixtures showed the lowest breakdown peak viscosity, indicating a reduction of starch content. Gel strength results showed that the addition of the oat-drink residue flour yields a softer gel texture, which translated to a softer crumb texture in the final baked products. Analysis of bread samples showed that oat-drink residue flour supplementation resulted in increased bread loaf volume at 10% inclusion levels, while bread samples showed volume reduction as the oat-drink residue flour concentration increased. Colour analysis revealed that the lightness (L*) of the crust and crumb of the bread samples decreased from DFOR1 to DFOR3, as oat-drink residue flour supplementation increased from 10 to 20%. Incorporating oat drink residue has the potential to be utilized in bread preparation in the context of sustainability in food production. Results highlight the potential for incorporating fractionated oat drink residue in modifying the properties of wheat flour for bread-making applications and improving the sustainability of the oat drink production process.

The effect of aging on chemical and organoleptic parameters of monastrell wines

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ABSTRACT

Fondillón is one of the most valuable naturally sweet Spanish wines, which is produced within the Alicante Protected Designation of Origin, (Alicante, PDO) and within the European Union in its E-Bacchus database. The present study was performed by analyzing four Alicante wine types, which were Fondillón (1988), reserva (2017), crianza (2018), and young (2020) with a different aging period in oak barrels but produced using only one variety of grapes, "Monastrell". The objective of the study was to evaluate the effect of the aging time on the chemical components and color characteristics of wine. In this context, anthocyanin compounds, trans-resveratrol, and colorimetric characteristics of the wines were analyzed. Anthocyanin compounds and trans-resveratrol were identified and quantified using LC/MS-MS, while the colorimetric characteristics [yellow-red-blue pigments, color intensity (IC), tonality (T), and color density (D)] of wines were measured by UV-Vis spectrophotometry. Fondillón wines with the highest aging time (>10 years) had the lowest total anthocyanin content (0.005 mg L-1), and this parameter decreased as the aging time increased, with contents being 1.877, 1.214, and 0.735 mg L-1 for young, crianza, and reserva "Monastrell" wines, respectively. Additionally, although trans-resveratrol was detected in young (0.146 mg L-1), crianza (0.619 mg L-1), and reserva (0.093 mg L-1) samples, this compound was not found at measurable contents in Fondillón. On the other hand, statistically significant differences were found among the values of color pigments, IC, T, and D for the wines under study and as affected by the aging time. Among the samples, Fondillón had the lowest percentage of red pigment (35.3%) but the highest percentage of yellow pigment (52.1%). As a result, a significant change was revealed in wine color from the initial red to the final brick red hue of Fondillón because of the decrease in anthocyanin and trans-resveratrol contents along the aging period.

Keywords: anthocyanins, colorimetric characteristics, Fondillón, trans-resveratrol

Physicochemical, pasting and thermal properties of water chestnut starch xanthan gum complexes as influenced by the addition of sucrose at different concentrations

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ABSTRACT

Effect of sucrose on swelling power, water absorption, freeze thaw stability, thermal and pasting properties of native water chestnut starch (WCS) alone and in the presence of xanthan gum (XG) was studied. Thermal and pasting properties were measured by using DSC (Differential Scanning Calorimeter) and Brabender Viscoamylograph respectively. The concentrations of sucrose used in the study were ranged from 10 to 30% due to the common concentration range applied in food products. Total polysaccharide concentration at 5% w/w of water chestnut starch –xanthan gum dispersions (at mixing ratio of 9.7/0.3). It was found that swelling power, freeze thaw stability of both WCS alone and in WCS/XG complex increased in the presence of sucrose at concentration below 30% while, water absorption was decreased. In the presence of sucrose a pronounced increase in paste viscosity of all WCS samples were observed. The onset, peak, and final temperature (To, TP, and Tf) of all water chestnut starch /xanthan dispersions with sucrose were found to be increased. The gelatinization temperatures (To, TP and Tf) and ΔH shifted significantly to higher temperatures with increasing sucrose concentration upto 20%.

Keywords: DSC, Sucrose, Viscoamylograph, Water chestnut, Xanthan gum

Knowledge, attitudes and dietary practices of health professionals regarding sustainable diet

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ABSTRACT

Sustainable diets are defined by the Food and Agriculture Organisation of the United Nations (FAO) as "diets with low environmental impact that contribute to food and nutrition security and healthy lives for present and future generations". Health professionals play a central role in the prevention and treatment of non-communicable diseases. They should also play a central role in promoting healthy and sustainable eating habits. The aim of the study was to investigate the attitudes and dietary habits, as well as the level of knowledge of health professionals regarding the environmental impact of food production and consumption. The study was conducted in 2021 and was designed as a cross-sectional study. Data were collected through an online self-report questionnaire from 103 health professionals of both genders with different levels of education and training backgrounds in nutrition and dietetics. Of the respondents, 66% indicated that they had some knowledge of sustainable nutrition. Respondents associated the concept of sustainable diets with low environmental impact (72.8%), healthy diets (66%), foods that are free of genetically modified organisms (GMOs) and pesticides and pest control agents (58.3%), and locally produced foods (53.4%). Regarding the obstacles to a sustainable diet, 79.6% of respondents said that there is a lack of sufficient knowledge on the subject, that it is difficult to find sustainable products (38.8%) and that the price of these products is too high (35%). Furthermore, 95% of the respondents would like to learn more about this topic, especially through a website dedicated to this topic (59.2%). Our research has shown that health professionals' knowledge about sustainable nutrition is insufficient, but that most respondents have a positive attitude towards this topic. To ensure greater engagement of health professionals in promoting sustainable dietary patterns, specially tailored and well-designed training programs are warranted.

Keywords: healthcare professionals, knowledge, sustainable diet

Total phenolic content and antioxidant properties of citrus peel and pulp

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ABSTRACT

Citrus fruits are recognized as a rich source of bioactive compounds such as polyphenols, carotenoids, vitamin C, and fibers with strong health-promoting effects. Some beneficial compounds are found in parts of citrus fruits that are not commonly used in the diet. The aim of this study was to compare total phenolic content (TPC) and antioxidant activity in different citrus compartments, i.e., peel and pulp, using two species from the family Rutaceae. Total phenolic content was analyzed by the spectrophotometric method at a wavelength of 630 nm. Samples were extracted with purified water to measure antioxidant activity by in vitro assays (DPPH, FRAP and CUPRAC). The composite antioxidant index (ACI) was then calculated to estimate the total antioxidant capacity.

The highest TPC was found in lime peel followed by lemon peel (584.6 ± 9.32 and 322.4 ± 12.70 mg GAE/100g, respectively; $p<0.01$) while the TPC in the pulp between lemon and lime was not significantly different. All the in vitro tests performed showed the highest antioxidant activity for lime peel and then for lemon peel. The ACI value was highest for lime peel (100%) and the lowest value was obtained for lime pulp (41.7%). Overall, there was a strong positive correlation between TPC and ACI ($r = 0.96$). Our results show that the outer part of citrus fruits has a higher concentration of antioxidant compounds than the inner part, suggesting that citrus peels can be used as a source of functional ingredients to produce new value-added food products or natural dietary supplements.

Keywords: citrus fruits, polyphenols, antioxidant activities

Antimicrobial, antioxidant and phytochemical properties of *citrus maxima* (pomelo) peel extracts

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ABSTRACT

Food preservation is a crucially important issue in the food industry. Chemosynthetic preservatives are widely used in the food industry against microbial contamination or oxidation. Awareness about utilizing "chemical-free" foods becomes popular and consumer demands are transformed into more conscious ones. To respond to this demand, alternative natural bio-active compounds obtained from food wastes must be investigated in detail. In this study, peels of *Citrus maxima* (pomelo), were used as raw material for obtaining bioactive extract. During processing of pomelo, significant amount of waste or byproducts, such as peels, seeds, and pulp, which comprise about 50% of fruit's original weight are generated (Anwar et al., 2008). These byproducts might be a useful source for substances used in functional foods, like flavonoids, dietary fibers, and essential oils (Senevirathne et al., 2009) or food preservatives against microbial contamination or as antioxidative agents. Bioactive extracts were obtained from pomelo peel by rotary evaporation technique in this work. Methanol (CMM) and acetone (CMA) (80%, v:v) were used as extraction solvents. Antimicrobial activities of CMM and CMA extracts of pomelo peel were tested against *Bacillus cereus* ATCC 11778 strain by agar well diffusion method. Anti-quorum sensing activities of CMM and CMA (inhibition zones: 8.5 and 10 mm, respectively) were investigated via violacein inhibition assay by using *Chromobacterium violaceum* ATCC 12472 strain. Total phenolic (121.4 and 140.7 mg mL^{-1}), and total flavonoid contents (1.7 and 2.1 mg mL^{-1}) of CMM and CMA extracts were determined respectively. Antioxidant activities of CMM and CMA extracts (64.9 and 67.8 % for four-fold diluted extracts, respectively) were monitored by DPPH free radical scavenging. In conclusion, CMM and CMA were determined as antimicrobial, antioxidant and phytochemically rich extracts which have potential to be used in food preservation.

Keywords: Antimicrobial, anti-quorum sensing, pomelo peel extract, phytochemical content, antioxidant activity

Nutritional composition and fatty acid profile of red goji berry (*lycium barbarum*) cultivated in Serbia

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ABSTRACT

Fruits of red goji have been used for centuries in Asian countries due to the high nutritive and non-nutritive bioactive compounds with potential health benefits. Cultivation and consumption of these berries have received worldwide attention in recent years, including Serbia. The study aimed to analyze and compare the nutritional composition and fatty acid profile of lyophilized red goji berries (*Lycium barbarum* L.) from six localities in Serbia. Nutritive compounds, such as moisture, ash, proteins, lipids, dietary fiber, and available carbohydrates, were determined using standard AOAC methods. The fatty acid profile was carried out using gas chromatography with a flame ionization detector, while polyunsaturated/saturated fatty acid ratio, atherogenic and oxi-disability index were calculated. The study results indicate a high content of available carbohydrates, proteins, and total fibers, with statistically significant variations among analysis berries from different localities ($p<0.05$). Expected differences between samples can be explained by various climatic and soil conditions, as well as pre-and post-harvest factors. Among identified fatty acids, the most abundant in all samples was linoleic acid, followed by oleic, palmitic, and stearic acid. All analyzed samples had a low atherogenic index and favorable PUFA/SFA ratio. Obtained results suggest that red goji cultivated in Serbia may be a source of well-balanced nutritive compounds with promising health effects on humans.

Keywords: *Lycium barbarum*, nutritional composition, fatty acids

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Investigation of antimicrobial effect of hazelnut green husk ethanolic extract

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ABSTRACT

Plants include phytochemical substances that have antioxidant and antimicrobial activities. Hazelnut (Corylus avellana) is one of the most popular nuts worldwide. In hazelnut processing industry over 300 thousand tons of green husk waste is generated annually. In the study, the extract of hazelnut green husks obtained by using ethanol, 80%, (v/v). Total phenolic content of the extract (76.7 mg GAE/g) was determined by using Folin-Ciocalteu method. Total flavonoid content (1.8 mg QE/g) was determined by using aluminum chloride method. Antioxidant effect of the extract (with 1/25 dilution) was determined by DPPH radical scavenging activity (%95). In addition, antimicrobial activities of the extract was determined by using agar well diffusion method against *Bacillus cereus* ATCC 11778, Staphylococcus aureus ATCC 29213, Streptococcus uberis ATCC 700407 and *Escherichia coli* ATCC 2592 with the inhibition zones of 19, 17, 16, and 7 mm, respectively. It was observed that the ethanol extract of hazelnut green husk was rich in phytochemical compounds and showed antioxidant and antimicrobial activities.

Keywords: Antimicrobial, antioxidant, hazelnut green husk, plant extract

The anti-biofilm effect of hazelnut green husk ethanolic extract

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ABSTRACT

Biofilm refers to bacterial communities that adhere to different surfaces and each other inside a matrix. Bacteria in biofilm matrix are known to be more tolerant to antimicrobial agents compared to planktonic bacteria. Chlorine treatment is often preferred for eradication of biofilms in the food industry. However, disinfection with chlorinated compounds can leave toxic residues which are harmful to environment and human health. Natural antimicrobial and antibiofilm agents are being investigated as an alternative to disinfectants that leave toxic residues in the food industry due to their low-cost, non-corroded equipment and non-affected sensory values.In this study, the extract of hazelnut green husk (GHE) was obtained by using ethanol. The antimicrobial effects of the extract (15, 12, 10, 8, and 7 mm of inhibition zones) with different concentrations (1, ½, ¼, 1/8, and 1/16, respectively) were determined by using agar well diffusion method against *Staphylococcus aureus* ATCC 25923. The removal ratios of 48 h old S. aureus biofilms by using GHE (with 1/8 dilution) and chlorine (200 ppm) were 25% and 65% respectively. In addition, application of GHE inhibited S. aureus biofilms by up to %100 (with 1/8 dilution). Anti-quorum-sensing properties of GHE were also determined against *Chromobacterium violaceum* ATCC 12472. It was shown that GHE extract had antimicrobial, antibiofilm, and anti-quorum-sensing activities.

Keywords: Antibiofilm, anti-quorum-sensing, hazelnut green husk, plant extract

Effects of cold plasma applications on bioactive composition of foods

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ABSTRACT

Cold plasma technology has been widely used for biomedical purposes and polymer modification. It has also been regarded as a promising technology that can be used for the treatment of food products. Cold plasma can provide microbial and enzymatic inactivation due to the production of reactive species and ultraviolet radiation. In addition, it has been reported by several researchers that the phenolic and bioactive contents of food products could be increased by cold plasma treatment (Herceg et al., 2016; Keshavarzi et al., 2020; Rashid et al., 2020). It can also improve the yield and antioxidant activity (Bao et al., 2020b). The processing parameters, such as voltage, frequency, gas composition, application time, characteristics of the product and the set-up of the equipment utilized in the cold plasma treatment affect the extraction rate, yield, antioxidant capacity, and quality (Bao et al., 2020a; Ekezie et al, 2017; Pragna et al., 2019). Thus, cold-plasma assisted extraction can be regarded as a novel technology that can be used for the extraction of bioactive components from foods or food byproducts, and the combination of cold plasma treatment with conventional or novel extraction methods, such as ultrasound-assisted extraction can enhance the yield and antioxidant activity. This study focuses on the utilization of cold plasma technology in the extraction of bioactive components from foods and food byproducts. The effects of process parameters are also discussed.

Keywords: antioxidant activity, bioactive properties, cold plasma, phenolic content

Investigation of some chemical properties of yoghurts fortified with lyophilized purslane (*Portulaca oleracea* L.*)* during storage

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ABSTRACT
Purslane is an annual plant which contains omega (ω) fatty acids and phenolic compounds. Therefore, it is essential to find an application area for this valuable plant in the food industry. The aim of this study is to manufacture yoghurts fortified with lyophilized purslane in order to improve the functional properties of yoghurt and to determine some chemical changes of produced yoghurts throughout storage. For this purpose, a central composite design (CCD) was constructed by considering the lyophilized purslane concentration (0.25-0.375-0.5%), starter culture ratio (1-2-3%) and storage time (5-7-12 days) as processing parameters. 39 different yoghurts were manufactured in a local dairy processing plant according to the experimental design. Some chemical properties of yoghurt samples including total phenolic content, total antioxidant capacity, fatty acid profile and water syneresis were determined throughout storage at 4 ℃. The total phenolic content of the yoghurts were improved by the addition of lyophilized purslane and reached up to 36.94 mg/kg. The antioxidant capacity of fortified yoghurts were in the range of 0.20-9.78 µmol Trolox /100 g. The fortified yoghurts were enriched by linoleic acid (2.99-11.31%). The presence of lyophilized purslane slightly decreased the water syneresis of yoghurt samples. The optimal fortified yoghurt processing parameters were determined to be 0.46% lyophilized purslane, 3.41% starter culture, and 5 days of storage. The principal component analysis results revealed a separation with respect to storage time. The production of yoghurts enriched by lyophilized purslane is a promising technique that can improve the product diversity and functionality of dairy products.

Keywords: functional foods, omega (ω) fatty acids, phenolics compounds, purslane, yoghurt fermentation

Determination Of Antioxidant Potential, Phenolic, and Aroma Profile In *Juniperus drupacea*

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ABSTRACT

Juniperus drupacea is a genus of perennial plants in the *Cupressacea* family. The color content, total phenolic content, antioxidant activity (ABTS and DPPH), phenolic profile, total sugar content (glucose, fructose, sucrose), and aroma compounds of *Juniperus drupacea* were investigated in this study. Juniperus drupacea generally showed high phenolic and antioxidant potential in water extract. The antioxidant activity total phenolic content were determined in the fruit flesh of *Juniperus drupacea*, as 118,40 mM Trolox/kg DW of DPPH, 159,75 mM Trolox/kg DW ABTS, and 18504 mg GA /kg DW, respectively. The amounts of sugar content in *Juniperus drupacea* were 12,52 g/kg DW of sucrose, 9,70 g/kg DW of fructose, 15,18 g/kg DW of glucose DW, and 37,40 g/kg DW total sugar content, respectively. Ten phenolic compounds were determined in *Juniperus drupacea* by LC-DAD-ESI-MS/MS. In the fruit extract, eighteen aroma compounds were detected in water extract *Juniperus drupace*. While amentoflavone (26,64 mg/kg), methyl-biflavone (25,13 mg/kg), and catechin (18,95 mg/kg) were the most significant phenolic compounds, *4*-nonene (161,62 mg/kg), and *δ*-cadinene (10,91 mg/kg) were the most significant aroma compounds in fruit flesh. The fruit water extract has an important antioxidant activity and phenolic profile. In light of the findings, it is suggested that the different products can be assessed using *Juniperus drupacea* water extract.

Keywords: *Juniperus drupacea L.*, antioxidant potential, LC-MS/MS, aroma compounds

1. INTRODUCTION

Juniperus drupacea L. (Andız) belongs to the Cupressacea family and is a rich source of bioactive compounds with potential health benefits. This species, which spreads only in the Eastern Mediterranean (Syria, Lebanon) and Southern Aegean regions (Greece and Turkey) in the world, cannot be evaluated adequately despite its rich content and unique sensory characteristics (Adams and Demeke, 1993; Adams, 1997; Talhouk et al., 2001; Bergmeier, 2002; Akıncı et al., 2004; Koutsaviti et al., 2017; Yavuz and Yılmaz, 2017; Douaihy et al., 2017; Walas et al., 2019). There is no information in the literature on the annual production amount of the andız fruit, which is produced from the andız tree, and naturally only spreads in the southern parts of the Mediterranean and Central Anatolia regions (Antalya, Karaman, Konya, Mersin, Adana, Hatay, Osmaniye and Kahramanmaraş) in our country (Akıncı et al., 2004; Yavuz and Yılmaz, 2017). Although there are different sources about the subclassification within the Cupressacea family (Hart and Price, 1990), which has a very different structure from other conifers (Saxton, 1913; Little, 2016; Gadek et al., 2000) is; in some sources, Thujoideae, Cupressoideae, Juniperoideae (Phillips, E. 1941; Gülsoy and Özkan, 2013), in some sources four subfamilies Actinostroboideae, Thujoideae, Cupressoideae, Juniperoideae (Engler, 1919; Li, 1953; Dönmez, 2005; İzgi, 2011; Memiş, 2011). The genus Juniperus is included under the name of the Juniperoideae subfamily, which is among these two dominant views (Engler, 1919; Phillips, E. 1941; Li, 1953; Dönmez, 2005, İzgi, 2011). Although it was stated that this genus was handled many years ago (Elwes and Henry, 2014), the Juniperus genus was first addressed by Spach in 1841 by the difference in leaf structures (needles or scales) and Oxycedrus Spach. and Sabina Spach. It was stated that it was divided into two sections (Kayacık, 1980; Güler, 2002; Dönmez, 2005; İzgi, 2011).

Juniperus genus in the classification made by Endlicher in 1847; Caryocedrus Endl was examined in three sections, Oxycedrus Endl and Sabina L., and *Juniperus drupacea* were discussed in the Caryocedrus section. *Juniperus drupacea* (Figure 1: C) was named Arceuthos drupacea (Labill.) or Syria Juniper in some sources (Kaeiser, 1954; Adams and Demeke, 1993; Sarıbaş, 2000; Gültekin and Gültekin, 2006; Jalal et al., 2014), and in some sources Andız, Indız, Anduz, Andız Giliği, Andız Katranı (Baytop, 1994), Toros juniper (Akkemik, 2017), Bear juniper (Kolosova et al., 2017), Habhel (Melbourne, 1876; Elwes and Henry, 2014), Duffran (Elwes and Henry, 2014), ar'ar (Shahat, 2019), Juniper, Juniper geliği, Juniper giliği, Bear giliği, Thorn Andız, Pıt Andız, Selbandız and Selbi Andız (Çakır, 2017).

A)  *B)*  *C)* 

Figure 1: *Juniperus drupacea Lab. (A)* Raw *(B)* Unripe *(C)* Ripe

The ripening quality (Figure 1) of the fruits used in food production contributes to the nutritional quality of food (Karababa and Işıklı, 2005). Also, many bioactive components found in andız fruit are sensitive to parameters such as species differences, growing conditions, and ripening stage, and they vary depending on these parameters (Evergetis et al., 2016). The fruit, which initially has a greenish color, turns from blue-violet to brown with ripening (1-2 years) (Hahn and Hahn, 2003; Akıncı et al., 2004; Turhan et al., 2007; Semiz et al., 2007; Güvendiren, 2015; Sobierajska et al., 2016). Plants produce phenolic compounds as secondary metabolites, widely distributed in various higher plant organs such as vegetables and fruits, playing significant roles in diverse physiological processes such as plant quality, coloring, flavor, and stress resistance. The phenolic compounds are natural antioxidants that have shown bioactivities, such as antioxidant, anti-microbial, anti-inflammatory, and anti-proliferative activities. When we were consumed phenolic compounds in enough doses regularly, prevention of various effects such as human diseases, including cardiovascular diseases and cancer (Zhang et al., 2022).

Over the years, the phytochemistry of the Juniperus genus has been studied; however, few reports focused on *J. drupacea* berries are available. Investigations on the berries of *J. drupacea* have led to the isolation and identification of monomeric tannin compounds, procyanidin dimers, phenolic acids, and flavonoids (Sakar, 1985; Sakar and Engelshowe, 1985; Miceli et al., 2011). Recently, the volatile chemicals and aroma compounds of Syrian *J. drupacea* berries have been analyzed (El-Ghorab et al., 2008; Vichi et al., 2007; Safkan, 2021). And there are published data concerning the characterization of phenolic and flavonoid compounds in *J. drupacea* berries (Miceli et al., 2011; Ereli, 2021). There are limited studies in the literature on the antioxidant activity, sugar content, color parameters, phenolic profile, and aroma compounds of *Juniperus drupacea*, in water extract. Characterization of bioactive properties and determination of antioxidant properties of water extract of *Juniperus drupacea* fruit were aimed to illuminate this study. The folin-ciocalteu method used to assess total phenolic content and antioxidant activity was determined using two (DPPH and ABTS) different methods. While phenolic compounds were determined using the LC-ESI-MS/MS, aroma compounds were determined using the GC-O-MS/MS.

2. MATERIAL AND METHODS

2.1. Sample and Chemicals

Juniperus drupacea fruits were purchased from a local market in Mersin, Turkey. The following HPLC standards were obtained from Sigma-Aldrich: chlorogenic acid, glucose (50-99-7), fructose (57-48-7), and sucrose (57-50-1) (Steinheim, Germany). Moreover, formic acid (Gernsheim, Germany), DPPH (2,2-Diphenyl-1 picrylhydrazyl), and ABTS (2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) reagents for

antioxidant analysis and total phenolic content were obtained from Sigma- Aldrich (Steinheim, Germany) and Folin–Ciocalteu's phenol reagent were obtained from Merck (Darmstadt, Germany). All chemicals and solvents were analytical or HPLC grade.

2.2. Extraction conditions

Ripe *Juniperus drupacea* fruits (~600 g) were bought from a seller in the Silifke region of Mersin province and brought to the food laboratory of Adana Alparslan Türkeş Science and Technology University. Fruit flesh used in this study was separated with a knife. The materials were ground in a household coffee grinder prior to analysis. Four grams of granulated andız fruit were weighed, 40 ml of room temperature water was added, boiled, and prepared with a coffee machine (~1 minute). After centrifugation at 6,500 rpm for 15 minutes at 4 ºC, the samples were filtered through a 0.45 m cellulose acetate filter (Millipore) before HPLC analysis (HPLC).

2.3. General Composition Analyses

The color was all analyzed in granulated and water extract *Junipperus drupacea* (Uzuner et al., 2011). The extraction of *Juniperus drupacea* was analyzed for sugar content according to the method described. For analysis, a 4 g sample was taken and then boiled with 40 ml of water (for ~1 minute 6 seconds), centrifuged at 6500 rpm at 4 °C. The supernatant was filtered through 0.45 µm membrane filters (Whatman Inc., Clinton, NJ, USA). Then, the extract was directly injected into HPLC to determine the sugar content of the samples. Analysis of the sugar content was performed by HPLC (Agilent 1260 HPLC system, CA, USA). A flow rate and an eluent were determined to be 0.5 ml/min, and 5 mM H_2SO_4, respectively. To determine the relationship between peak area, and concentration, a calibration curve was created using (sucrose, glucose, and fructose) standards (Lee and Coates, 2000).

2.4. Antioxidant activity and total phenolic content analyses

2.4.1. DPPH assay

The DPPH assay was determined by the method previously described by Brand-Williams et al. (1995) with modifications. In a brief, 100µl of *Juniperus drupacea* water extract was mixed with 3.9 ml of DPPH solution and incubated at room temperature for 30 minutes in the dark. Using a UV–visible spectrophotometer, the absorbance was measured at 515 nm (Carry 60, Agilent, Malaysia). The antioxidant activity was expressed as Trolox equivalents per 100 grams.

2.4.2. ABTS assay

The antioxidant capacities of the samples were determined by the ABTS method previously described by Saafi et al (2009). The antioxidant activity was calculated using a calibration curve and represented as Trolox equivalents per 100 gram.

2.4.3. Total phenolic content assay

Total phenolic content was determined with minor modifications in a Folin method previously described by Galvão et al., (2018). TPC was calculated as gallic acid equivalents and quantified in milligrams per 100 gram of extract (GAE).

2.5. Determination of the phenolic profile of *Juniperus drupacea* by LC/ESI-MS/MS

Determination of phenolic compounds was made by LC-MS/MS, according to Kelebek (2016). A Phenomenex Luna reverse phase C-18 column (4.6 mm 250 mm, 5 m) was utilized in the HPLC system. Water/ formic acid A (99:1; v/ v, Solvent A) and methanol/ formic acid A (60:40; v/ v, Solvent B) were employed as mobile phases, with flow rates of 0.5 ml/ min and temperatures of 25°C, respectively. An Agilent 6430 LC–MS/MS spectrometer equipped with an electrospray ionization source was used to confirm the identification of each phenolic component by comparing its retention index and UV spectra to pure standards. In negative ion and MRM modes, mass spectrum data of phenolic compounds were obtained for identification and quantification.

2.6. Determination of the aroma profile *Juniperus drupacea* by GC-FID and GC-MS

The aroma compound was determined by the method described by Selli et al. (2014) with modifications. *Juniperus drupacea* aroma for gas chromatography-mass spectrometry (GC-MS) analysis will be extracted by the head-space solid phase micro-extraction (HS-SPME) method. The gas chromatography (GC) system consisted of an Agilent 6890 chromatograph equipped with a flame ionization detector (FID), an Agilent 5973-Network179 mass selective detector (MSD) (Wilmington, USA). This system allowed us to simultaneously obtain an FID signal for the quantification and an MS signal for the identification. GC effluent was split 1:1 among the FID, and MS. Aroma compounds will be separated on a polar DB–Wax column (60 m length × 0.25 mm i.d. x 0.4 μm thickness, CA, USA). Helium, a carrier gas, will be employed with a flow rate equal to 1.2 mL/min. The temperature parameters will be set up as follows: the initial temperature of the oven will be 40 °C for 4 min, the next increase rate is 3 °C min^{-1} up to 130 °C, and that temperature will be kept constant for 4 min. Afterwards, another temperature ramp will be applied with a rate of 5 °C min^{-1} up to 240 °C. Finally, it will be kept at this temperature for 8 min. The electron ionization mode: 70 eV and m/e series: 30–300 amu at a scan rate of 2.0 scan s^{-1}. All aroma compounds will be identified by the mass spectral database (NIST 11L, Wiley 7, Flavor 2L), retention index, and chemical standards. After identification, calculation of each aroma compound concentration will be performed by FID based on *4*-nonanol (with a concentration of 41.5 mg/kg) equivalents as the internal standard. The internal standard method will be conducted to quantify the volatiles. *4*-Nonanol will be used as an internal standard in the extractions. A combination of experimental calibration by internal standards and FID response factors prediction will be carried out. Therefore, quantification will be calculated based on *4*-Nonanol concentration.

3. RESULTS AND DISCUSSION

3.1. Chemical Composition of *Juniperus Drupacea*

Table 1 shows the chemical and color properties of *Juniperus drupacea*, expressed as means, which correspond to the three analytical replicates. As can be seen in Table 1, dry matter, color content (extract and dry sample), and sugar content of *Juniperus drupacea* were found as 83.79%, L* (74.46 and 38.73), a* (13.00 and 13.59), b* (61.50 and 15.71), c* (62.86 and 20.77), H (78.06 and 49.24), and total sugar 37.40 g/kg, respectively. According to results of earlier work on Andız molasses (İzgi, 2011; Erbil, 2020; Ereli, 2021), color content and total sugar contents were found between L* (2.64-49.78), a* [(-0.05)- 24.48], b* (0.62-38.57), c* (15.53-39.70), H (15.95-76.31) and 6.61-54.72%, respectively.

Juniperus drupacea color is the most important external characteristic to asses ripeness and postharvest life. While the L value represents brightness (100 points whiteness or lightness/ 0 points darkness ratio), a* is (+/-) red/green, and b* (+/-) yellow/blue. L* (2.64-49.78), a* [(-0.05)- 24.48], b* (0.62-38.57), c* (15.53-39.70), H (15.95-76.31) and 6.61-54.72%, respectively. Chromaticity values of andız fruit for dry samples (L*, a*, b*, c*, and H) were very similar to those reported by İzgi, 2011; Erbil, 2020; Ereli, 2021.

Sugars are the major components of the soluble solids in Andız extract and the sweetness of extract is intrinsic to its sugar composition. The main sugars in the Andız extract were sucrose, fructose, and glucose, with glucose predominating (Table 1). The total amount of sugar in the studied sample was 37.40 g/kg. Total sugar content in Andız molasses has been reported (İzgi, 2011; Erbil, 2020; Ereli, 2021); however, in the other studies, the unit did not give the same in our study. However, there is no knowledge about the sugar content of *Juniperus drupacea* water extract in the literature.

Table 1. Chemical composition, antioxidant capacities, total phenolic contents of *Juniperus drupacea* water extract

ANDIZ (*Juniperus drupacea*)		**Quantity**
DRY MATTER	(%)	83,79±0,14
Color (Extract)	L*	74,46±0,03
	a*	13,00±0,01
	b*	61,50±0,02
	c*	62,86±0,02
	H	78,06±0,00
COLOR (Dry Sample)	L*	38,73±0,01
	a*	13,59±0,01
	b*	15,71±0,02
	c*	20,77±0,02
	H	49,24±0,22
SUGAR (g/kg)	Sucrose	12,52±0,12
	Glucose	15,18±0,46
	Fructose	9,70±0,00
	Total Sugar	37,40±0,58
Total Phenolic Content	*Folin-Ciocalteu* (mg GAE/kg)	18504±0,3
Antioxidant Capacities	*DPPH* (mmol Trolox/kg)	118,40±2,83
	ABTS (mmol Trolox/kg)	159,75±2,9

3.2. Antioxidant Capacities and Total Phenolic Content of *Juniperus Drupacea* Extract

The antioxidant capacity of Andız fruit flesh water extract was evaluated by DPPH and ABTS assays. It showed the scavenging activities of Andız water extracts on the DPPH in Table 1. The antioxidant capacity of the sample was analyzed by the DPPH method and found to be 118,40 mM Trolox/kg DW. The ABTS value in *Juniperus drupacea* was 159,75 mM Trolox/kg DW. There are studies on antioxidant capacity in the literature on andız or andız molasses (Taviano et al., 2011; Yüksel, 2013; Temel, 2014; Ereli, 2021). However, the processing steps (as a unit) in the conclusion phase of the findings discussed in the research are not the same, so a comparison with our results could not be made.

The Folin Ciocalteu, one of the most frequently used methods, was employed to quantify total phenol content (TPC). The TPC value of Andız extract was 18504 mg GAE/kg DW. The total phenolic amounts determined in the studies on andız in the literature; Yüksel (2013) 892.57-3968.37 mg/L, Akbulut et al. (2008) 96.5 (g/kg), Özdemir et al. (2014) 1600-2070 mg/kg, İzgi (2011) 949-2100 mg/kg, Sarıaydın et al. (2014) 4.98-30.38 (g/kg), Miceli et al. (2011) 48.06 mg GAE/g, Taviano, et al. (2011) 184.23 GAE/g and 98.74 mg GAE/g, Ereli (2021) 111.79-230.77 mg GAE/kg DW, Orhan et al., (2019) 122.56 mg GAE/g, 340.67 mg GAE/g in ethyl acetate extract and 85.93 mg GAE/g in water extract reported. When we compared results, this study showed total phenolic contents stronger than the literature studies.

3.3. Identification of Phenolic Compounds By HPLC-DAD-ESI-MS Analysis

Phenolic profiles of *Juniperus drupacea* were identified by LC-DAD-ESI-MS/ MS analysis (see Table 2). Ten phenolic compounds were determined in *Juniperus drupacea* by LC-DAD-ESI-MS/MS. Gallic acid, tyrosol, *4*-hydroxybenzoic acid, *p*-hydroxybenzoic acid, vanillic acid, dihydroxy benzoic acid, catechin, digalloylquinic acid, amentoflavone, and methyl-biflavone were observed in the *Juniperus drupacea* water extract (Figure 1). Individual compound quantification was determined using standard compound calibration curves. The curves were created using commercially available standards for the amounts found in Andız extracts.

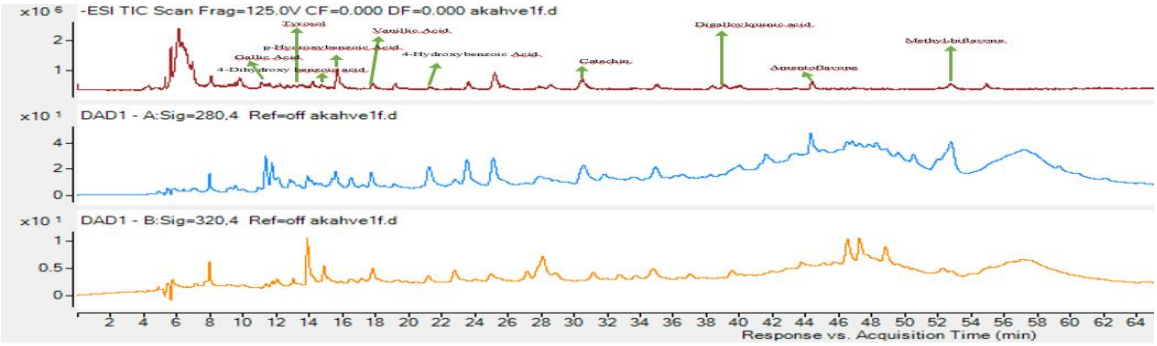

Figure 1. HPLC chromatograms of *Juniperus drupacea* extract were recorded at 280, and 320 nm. The compounds corresponding to peaks are listed in Table 2.

Flavones and flavonols (flavus-Latin for yellow) are present in plants as secondary metabolites. They are naturally yellow in color. The chemical structure has a 15-carbon skeleton, with two phenyl rings (A and B) and one heterocyclic ring (C); abbreviated as C6-C3-C6. They are anthoxanthins (flavones and flavonols), and ketone-containing polyhydroxy polyphenol compounds. There are natural flavonoids of more than 5000. Flavonoids are known for their diverse biological activities, such as antibacterial, antiviral, antifungal, antimicrobial, antidiarrheal activities, anti-inflammatory, and antioxidant (Kurhekar, 2020).

Table 2. Main phenolic compounds and quantities (mg/kg) of *Juniperus drupacea* water extract by HPLC-DAD-ESI-MS/MS, including retention time, λmax in the ultraviolet region (280 nm), molecular ion, quantitative transition (*m/z*), and tentative identification

Peak No	RT (min)	[M-H]⁻ (m/z)	Compound	Quantity	References
1	11,707	290-169-125	Gallic Acid	4,49±0,01	1,2
2	13,83	137-93	Tyrosol	1,73±0,00	1,2
3	15,48	137-93	*4*-Hydroxybenzoic Acid	4,71±0,01	-
4	16,42	137	*p*-Hydroxybenzoic Acid	4,12±0,01	1
5	17,57	167-152	Vanillic Acid	6,57±0,01	1
6	20,83	153-109	Dihydroxy benzoic acid	10,30±0,02	-
7	30,21	289-245	Catechin	18,95±0,00	2
8	39,93	495-343	Digalloylquinic acid	8,72±0,05	-
9	44,32	537	Amentoflavone	26,64±0,01	1,2
10	52,79	551	Methyl-biflavone	25,13±0,00	1,2
		Total Quantity		111,35	

(1) Ereli, (2021): (2) Miceli et al., (2011)

Regarding individual flavonols, the major compounds found in *Juniperus drupacea* were amentoflavone and methylbiflavone. Amentoflavone, a natural biflavonoid compound. Some researchers also confirm that Amentoflavone exhibits an enormous antioxidant ability by inhibiting the production of hydroxyl radicals, superoxide, ABTS and DPPH in a variety of free radical scavenging models in vitro (Xiong et al., 2021). While the mass spectrum of amentoflavone and methyl-biflavone was producing the predicted molecular ion at m/z 537 $[M-H]^-$, and at m/z 551 $[M-H]^-$ (Table 2). Of all these compounds, amentoflavone, methyl-biflavone, and catechin is the predominant compound in the *Juniperus drupacea* water extract, the amount of which is 26.64 mg/kg DW, 25.13 mg/kg DW, 18.95 mg/kg DW, respectively. In some studies, gallic acid, tyrosol, catechin, amentoflavone, and methyl-bi-flavone have been reported as the dominant flavonol compound in *Juniperus drupacea* (Miceli et al., 2011 and Ereli, 2021), the amount of which was 1016 µg/g extract, 1324 µg/g extract, 181 µg/g extract, 927 µg/g extract, and 660 µg/g extract, respectively (Miceli et al., 2011). Gallic acid (2.38-14.23 mg/kg), tyrosol (2.83-8.31mg/kg), *p*-hydroxybenzoic acid (8.32-11.48 mg/kg), vanillic acid (8.26-10.36 mg/kg), dihydroxy benzoic acid (2.82-5.28 mg/kg), amentoflavone (2.13-12.54 mg/kg), and methyl-biflavone (2.24-11.04 mg/kg) was found by Ereli (2021). The total phenolic compound content determined in our study is similar to other studies (Ereli, 2021).

3.4. Aroma profile with HS-SPME in *Juniperus drupacea* separation by GC-FID and GC-MS

The volatile compounds identified in *Juniperus drupacea* and these compounds were presented in Table 3. Mean values (bmg/kg) of the GC analyses of triplicate extractions and standard deviations were reported. As shown in Table 3, a total of 18 compounds was identified and quantified in *Juniperus drupacea*, most of which have already been identified by previous studies (Safkan et al., 2021; Vichi et al., 2007; El-Ghorab et al., 2008; Koutsaviti et al., 2017). However, seven aroma compounds (*4*-nonene, toluene, *4*-nano none, *m*-Methylstyrene, benzoic asit, azulene, phenanthrene) were identified and reported for the first time in *Juniperus drupacea*.

Table 3. Aroma compounds of *Juniperus drupacea* water extract

No	RT	Aroma Compounds	Concentration (mg/kg)	Group	References
1	**3,667**	***4*- nonene**	**161,62±0,06**		-
2	7,488	Toluene	3,00±0,01		-
3	9,312	*p*-xylene	1,18±0,00	Terpenes	*A*
4	12,548	*dl*- limonene	6,88±0,02	Monoterpenes	*A, D*
5	15,829	*p*-cymene	1,71±0,06	Monoterpenes	*B, D*
6	19,249	*4*-nanonone	4,32±0,00		-
7	25,256	*m*-Methyl styrene	6,00±0,04		-
8	33,772	benzoic acid	2,49±0,00		-
9	34,649	*γ*-muurolene	8,98±0,09	Sesquiterpenes	*B, C*
10	35.866	*α*-muurolene	2,24±0,02	Sesquiterpenes	*B*
11	**37.017**	***δ*-cadinene**	**10,91±0,42**	Sesquiterpenes	*B*
12	38.478	hexanoic acid	3,53±0,03	Acids	*A*
13	39,817	cadina-1,3,5-triene	3,46±0,08	Terpenes	*B*
14	42.036	*α*-calacorene	2,75±0,07	Terpenes	*B*
15	46,288	nonanoic acid	5,18±0,09	Acids	*A*
16	46,509	*β*-cubebene	3,71±0,05		*B, D*
17	47.071	Azulen	6,99±0,09		-
18	50,268	Phenanthrene	7,06±0,07		-
Total aroma compounds			**283,52**		

(A) Safkan et al. (2021); *(B)* Vichi et al. (2007); *(C)* El-Ghorab et al. (2008); *(D)* Koutsaviti et al. (2017)

The major components in the volatile fraction of *Juniperus drupacea* were determined as *4*-nonene (161,62 mg/kg) and *δ*-cadinene (10,91 mg/kg). The *Juniperus drupacea* water extract had 283,52 mg/kg aroma compounds, which generally included terpenes compounds. *p*-xylene (123 µg/L), *dl*- limonene (279 µg/L), hexanoic acid (928 µg/L), and nonanoic acid (118 µg/L) were determined by Safkan et al. (2021). When we compared with results previously obtain, researchers show that terpenoids compound abundance of *Juniperus drupacea* (Safkan et al., 2021; Vichi et al., 2007; El-Ghorab et al., 2008; Koutsaviti et al., 2017). These results are in agreement with those obtained previously reported by other authors (Table 3).

5. CONCLUSION

In this study, *Juniperus drupace* extract includes sucrose, fructose, and glucose, with glucose predominating. This study used two different methods (DPPH and ABTS) to assess the antioxidant properties of *Juniperus drupacea* water extracts. With the testing methods, all the extracts exhibited strong antioxidant potential. Overall, the *Juniperus drupacea* extracts were highly effective in antioxidant capacity in our study. The ten phenolic compounds in the sample were identified by LC-DAD-ESI-MS/MS. Amentoflavone, methyl-biflavone, and catechin are the major phenolic compounds. The eighteen aroma compounds in the sample were identified by GC-FID and GC-MS. *4*-nonene and *δ*-cadinene are the major aroma compounds. *Juniperus drupacea* is a fruit that is not usually consumed directly despite its rich components. With this study, the antioxidant potential and phenolic content of Juniperus water extract were shed light, and it was observed that it had strong antioxidant potential, phenolic content, and aroma compound. In light of the findings, it is suggested *that Juniperus drupacea* fruit should be consumed/evaluated. In light of the findings, it is suggested that the different products can be assessed using *Juniperus drupacea* water extract. The different beverages can be produced using *Juniperus drupacea* aroma.

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Characterization of volatiles and key odorants of Akpi (*Recinodendron heudoletii*) nuts as affected by single and double roasting process

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ABSTRACT

Raw *Recinodendron heudoletii* and its roasted nut samples, obtained from single (10 min) and double (20 min) roasting, were subjected for the first time to volatile substances and powerful odorants analysis through Gas Chromatography Mass Spectromety Olfactometry (GC-MS-O) technique. The isolation of volatile substances was performed by purge and trap method, and 30, 41, 39 aroma were detected in raw, single and double roasted nut samples, respectively. Principal component analysis (Capcarova et al.) was applied to evaluate the correlation between roasting processes and raw aroma compounds of nut samples. By employing the aroma extract dilution analysis (AEDA), 18, 25, 23 were revealed in raw, single and double roasted nut sample, respectively. The most substantial differences between raw and roasted nut samples were as follow: 2,3-Dimethylpyrazine, 2-Methylpyrazine, 2-Acetylpyrazine, 2-Ethyl-5-methylpyrazine, 2-Acetyl-5-methylfuran, 5-Methyl-2-hexanone, Furfuryl alcohol, 2-Methylbutenal, Benzaldehyde were only determined in roasted samples while p-cymen was only detected in raw nut samples. Hexanal (green-like odor) was the most prevailing key odorant with flavor dilution factors (FD) of 128, 1024 and 256 in raw, single and double roasted nut samples, respectively. Recinodendron heudoletii nut samples were strongly characterized by polyunsaturated fatty acid, in which Eicosapentaenoic acid (C20:5) was found in higher amount in raw (51.05%) and single roasted (47.76%) nut samples, while Cis-13,16-Docasadienoic acid (C22:2) was prominent in double roasted (40.67%) nut samples.

Keywords: *Recinodendron heudoletii*, aroma, AEDA, GC-MS-O

The effect of heat moisture treated-banana flour addition as composite material of noodle on its post prandial glucose profile and noodle characteristics

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ABSTRACT

Noodles made of HMT banana flour (HBF) and wheat flour (WF) with the ratio 0 to 50% of MBF (**Fig 1**) have been characterized for their postprandial glucose response in mice, organoleptic, color as well as the cooking properties. Wheat flour substitution with HBF tends to decrease glycemic index of the noodle. Furthermore, the substitution with 50% HBF displays a more stable glucose level until the end of the observation (4 hours) suggesting the long lasting energy characteristics of the product. This result also indicates that noodle with 50% HBF contains higher slowly digestible starch. Substitution with HBF tends to decrease cooking time but increase cooking loss. No significant difference in the cooking yield is observed. In terms of color properties, the substitution alters color properties to be darker. The substitution also tends to decrease sensory characteristics of the noodles, particularly colour and taste. These drawbacks particularly the taste can be overcome when noodle is consumed with spice.

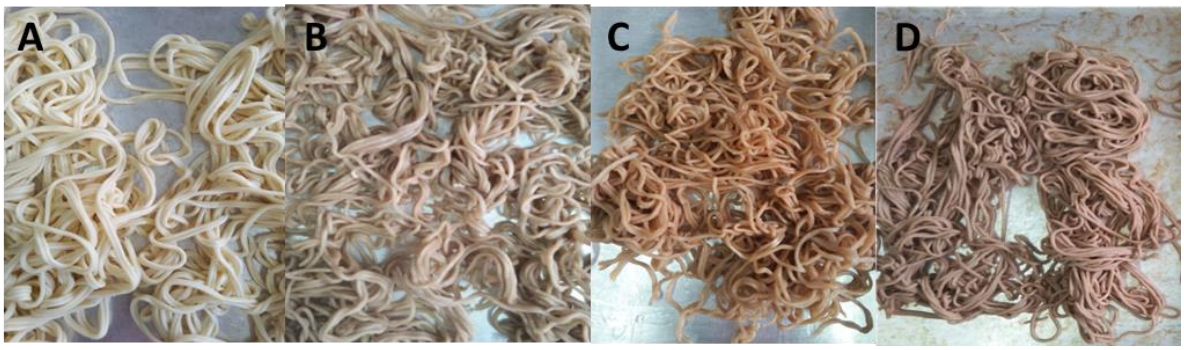

Figure 1. Noodles made of different ratio of HBF and WF

Keywords: Noodle, Heat moisture treatment, starch, postprandial glucose, slowly digestible starch

Characterization of probiotic candidate lactic acid bacteria isolated from "dadih" a fermented buffalo milk as biopreservation in beef

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ABSTRACT

As a perishable food, beef can be preserved naturally utilizing antimicrobial compounds through a process called bio-preservation. Lactic acid bacteria are probiotic bacteria or bacteria that can produce antibacterial metabolites. Lactic acid bacteria are found in fermented foods like "dadih," or fermented buffalo milk from West Sumatra, Indonesia. The aim of this study is to investigate the presence of probiotic lactic acid bacteria in "dadih" and the efficacy of using metabolites of lactic acid bacteria as bio preservatives in beef for nine days at a low temperature (4°C). The DK1 isolate demonstrated the highest antibacterial metabolite activity against *Escherichia coli* (13 mm) and *Salmonella* sp. (11.5 mm) of the three lactic acid bacteria isolates tested. The DK1 isolate was differentiated by its probiotic properties, with resistance test results of 0.3% bile salt, pH 2-4, temperature 25°C-45°C, and over 106 CFU/ml. 89.2% of the time, DK1 isolates tested positive for auto-aggregation. *Salmonella* sp. and *E. coli* co-aggregation test results show 46.9% and 53.1%, respectively. In general, treated beef had less *E. coli* and other bacteria than untreated meat, according to the bio-preservation results. Moreover, metabolite-treated meat displayed a lower and more consistent pH value as well as a color shift compared to control beef.

Keywords: Beef, bio-preservation, lactic acid bacteria, dadih

Spontaneous fermentation process of Ivorian cocoa beans and the microorganisms involved

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ABSTRACT

Cocoa (*Theobroma cacao*) beans are among the most important ingredient in food and beverage industries. It is mainly cultivated in tropical and subtropical forests. Africa is the biggest cocoa beans provider in the world market, in which Ivory Coast remains the world leader with an estimated yearly production of 2 million tonnes. Cocoa beans are used for chocolate products, cocoa butter, confectionery products, ice drinks, cocoa powder production. The quality and organoleptic characteristics of these products are strongly related to that of cocoa beans obtained from different processing steps. The pulps surrounding the beans are rich in water, sugars, pectins, proteins, minerals, vitamins, citric acid, and phenolic compounds. After harvested cocoa pods, many different cocoa beans processing steps are performed, among which fermentation is the first and crucial post-harvest step for the release of aroma and aroma precursors compounds and the end-products quality. Spontaneous fermentation is mostly carried out by the cocoa farmers in Ivory Coast and some African, Asia, and Latin American countries. The microorganisms involved in this stage are primary the yeasts (anaerobic phase), they convert the pulps into alcohol with a sporadical increase in temperature, and then the lactic and acetic acid bacteria (aerobic phase) into acetic and lactic acids. The degradation of the substracts inside the cocoa pulps allows the generation of aroma and aroma precursors. Generally in Ivory Coast, the fermentation is performed on banana leaves, boxes or heaps and starts at ambient temperature for 4-6 days. This paper aims to set an overview of Ivorian cocoa beans fermentation process, microorganisms involved, fermentation conditions, and aroma precursors release.

Keywords: Cocoa, fermentation, aroma precursors, microorganisms, spontaneous.

2T2D COS PLS-DA applied to multispectral imaging to discriminate beef muscles

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ABSTRACT
The aim the study was to evaluate the ability of Visible Near infrared multispectral (VIS-NIR MSI) imaging coupled with 2T2D COS PLS-DA (two-trace two-dimensional correlation spectroscopy and partial least squares discriminant analysis) to classify beef muscles based on their breed origin and muscle type. The experiment was performed on 240 muscles of three types (Longissimus thoracis, Semimembranosus, and Biceps femoris) of three breeds (Aberdeen Angus, Limousine, and Blonde d'Aquitaine). Before PLS-DA, the VIS-NIR MSI spectra were processed and transformed by 2T2D COS in order to calculate synchronous and asynchronous maps. The results highlighted that combining non-preprocessed synchronous and asynchronous 2T2D maps before performing PLS-DA was the best strategy to reach 100% of classification accuracy between muscles and breeds classes.

Keywords: beef, breed, muscle, discrimination, multispectral imaging, 2T2D COS, PLS-DA.

Changes of Biochemical Compositions of Blueberry Fruit During Shelf Life

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ABSTRACT
Effects of preharvest hexanal spray treatment of Star, Beloxi, Legacy, Blueribbon blueberry varieties biochemical compositions were determined during shelf life. For this purpose Star, Beloxi, Legacy, Blueribbon blueberry varieties were treated with hexanal spray applications which were, doses (0%, 0.02%) prior to one day before harvest sprayed then harvested fruits were stored at 2ºC and RH %90 conditions. Effects of hexanal spray applications of fruit physical and chemical composition were analyzed. Differences among treatments were identified and among treatments (0.02% hexanal and control) and cultivar and shelf life on anthocyanin, total phenol content and free radical scavenging capacity, sugar content of blueberry fruit storage at 2ºC and RH %90 conditions at initial time, 6 and 12 days during shelf life. Results showed that, hexanal application positively effected the biochemical composition of anthocyanin, total phenol content and free radical scavenging capacity, sugar content and weight loss and firmness during shelf life. The hexanal application were effected anthocyanin, total phenol content and free radical scavenging capacity amount of blueberry varieties. As a result, hexanal application has important effects on biochemical compositions of blueberry varieties during shelf life.

Keywords: blueberry, shelf life, hexanal spray, quality

Effects of Preharvest Hexanal Treatment on Chemical Compositions of Rasberry Fruit During storage

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ABSTRACT
Changes in anthocyanin, total phenol content and free radical scavenging capacity compositions and weight loss, brix, sucrose, glucose, xylose, fructose and total sugar content of raspberry were determined in Diamond Jubile, Jade rasberry varieties releated to pre harvest hexanal application during shelf life. Diamond Jubile, Jade rasberry varieties were treated with hexanal spray applications (0%, 0.02%) preharvest and stored at 2°C and RH %90 conditions. Effects of hexanal spray applications of rasberry fruit chemical compositions were analyzed in a spectrophotometer and determined by HPLC techniques. Differences among treatments were identified in chemical compositions at four days intervals during 10 days of storage life. Results showed that, hexanal application effected the amount of anthocyanin, total phenol content and free radical scavenging capacity, weight loss, brix, sucrose, glucose, xylose, fructose and total sugar composition during storage life. Finally, preharvest application were determined that signifant effects on chemical composition of rasberry during storage.

Keywords: rasberry, shelf life, hexanal spray, quality

Prototype unit for continuous manufacturing of milk tablets

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ABSTRACT

Milk tablets are easily ready-to-consume food items prepared from compressing milk powder with added benefits of handling, application, transportation, and storage. In the current research work, improvement in the skim milk powder (SMP) tableting technology was done by varying the moisture content (MC) of powder and depth of press (DP) of the lab-scale pressing machine. The full-factorial experimental design was made and response surface methodology (RSM) was used for optimizing tableting conditions with the objective to have the lowest solubility time (ST) and desired tensile strength (TS) of tablets. The improved technology and experimental design were used for developing novel milk tablets like whole milk powder (WMP) tablets and goat milk powder (GMP) tablets. Sugar was used as a natural disintegrant for further improvement in the quality of each milk tablet. The tableting study produced SMP tablets, WMP tablets, and GMP tablets with TS in the range of 74.47-283.69 kPa, 18.17-103.76 kPa, and 5.59-41.76 kPa respectively and ST in the range of 19.67- 317.53 s, 81.67-442.28 s, and 109.84-340.75 s respectively. RSM showed good fitness of cubic models for TS of SMP tablets, WMP tablets & GMP tablets, and ST of SMP tablets &WMP tablets, however, the quadratic model was best for ST of GMP tablets. Lowering of MC has little effect on TS of each tablet but a significant decrease in ST. In contrast, DP has a significant positive effect on both TS and ST. A further significant decrease in ST and TS of milk tablets was obtained from increasing sugar concentration. However, the sensory analysis showed the suitability of 10-11 % w/w, 14-15% w/w, and 14-15% w/w sugar concentration for SMP tablets, WMP tablets, and GMP tablets. At last, the developed technology of milk tablets is being scaled-up for mass production on an industrial scale with a focus on commercialization.

Keywords: SMP tablet, WMP tablet, GMP tablet, tensile strength, solubility time

Development of a Fermented Plant-based Product Structure from Pistachio

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ABSTRACT

Fermented plant-based products have gained popularity in last decades due to presenting a healthy alternative for consumers who cannot consume dairy products due to health reasons and for individuals who prefer to follow a plant-based diet. This study aimed to produce a fermented plant-based spreadable product structure from pistachio.

Peeled raw and roasted pistachios were mixed with water and sucrose and pasteurized. The slurries were fermented with a lactic culture for 5 h until the pH value was 4.5. Fermented slurries were mixed with locust bean and xanthan gum solutions to form a stable spreadable structure without phase separation. The real time rheological measurements were conducted to investigate how rheological properties of slurries changed with temperature and during fermentation in linear viscoelastic region. Rheological properties of the final product were measured by applying oscillation strain sweep test and frequency sweep tests. Textural properties of the product were determined by a back-extrusion test. Water holding capacity, soluble protein, pH and titratable acidity of the samples were also measured.

Heating and fermentation resulted in a continuous increase in the elastic and viscous moduli of the pistachio slurries. pH values decreased slightly to 4.36 in the sample prepared from raw pistachios and 4.33 in the sample from roasted pistachios on the 1st day after production. Corresponding titratable acidity values were found to be 0.31% and 0.29% lactic acid, respectively. While fermentation did not cause a significant change in soluble protein content of the sample with raw pistachios, fermentation decreased the soluble protein content in the sample with roasted pistachios. Roasting of pistachios resulted in lower yield stress, firmness and stickiness in the fermented product compared to the use of raw pistachios. There was no water separation in the samples after centrifugation. A better structure was obtained by fermentation of raw pistachios compared to that of the roasted ones. Further studies are required for determination of sensory properties and shelf life.

Keywords: Fermentation, Plant-based, Pistachio, Gel, Rheology, Texture

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Life cycle assessment analysis of novel dryer prototype: carbon fiber-assisted dryer

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ABSTRACT

Tomato pomace is an industrial waste of tomato paste and juice with rich nutrient content. Recycling and reusing tomato pomace is important for a sustainable approach. In this study, the novel dryer prototype named the carbon fiber-assisted dryer was constructed. The environmental impact of an alternative dryer design on the drying of tomato pomace was determined by life cycle assessment (LCA). In this context, 1 kg of dried tomato pomace production was taken as the unit basis. Tomato pomace was dried at three different temperatures (40°C, 50°C, and 60 °C) in two different dryers (a carbon fiber-assisted (CFA) dryer, and a tray dryer) up to final moisture content of 7%. The processing time in both dryers decreased as the applied temperature increased. The environmental impact was lower in CFA dryer with lower energy consumption. Accordingly, the emission values in the examined environmental categories were reduced by up to 50%. Global warming potential (GWP, kg CO_2 eq) values decreased as the drying temperature increased. For 40°C drying temperature, GWP values of the CFA dryer and the tray dryer were 58.88 kg CO_2 eq and 84.93kg CO_2 eq, respectively. In conclusion, the constructed dryer prototype is expected to be an alternative to the tray dryer and reduce the impact of drying tomato pomace in terms of sustainability. This study is financially supported by DİMES FOOD Ind. and Inc.

Keywords: warming, tomato , sustainability, drying, environment

Atmospheric cold plasma application on tomatoes

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ABSTRACT

Tomatoes have high export potential and have a limited shelf life due to their microbial load. With alternative food processing methods, the shelf life of tomatoes can be extended. Atmospheric cold plasma (ACP) is one of the novel non-thermal food processing technologies that can reduce quality losses and provide microbial inactivation. In this study, the effect of ACP application onthe exergetic spore reduction rate of *Alternaria alternata* spores ($X_{log,}$ log spores/kJ) and the antioxidant activity of tomato under different treatment times (5 and 20 min) and different voltage gradients (7 kV/mm, 13 kV/mm) were investigated. In both voltage gradients, the antioxidant activity values of the tomato decreased as the treatment time increased. Antioxidant activity content of tomatoes decreased by 28.33 ±1.27% after 20 min of ACP application. At the voltage gradient of 7 kV/mm, the X_{log} value decreased from 0.62±0.07 log spores/kJ to 0.24±0.01 log spores/kJ as the treatment time increased from 5 min to 20 min. A similar trend was obtained for the higher voltage gradient. In summary, the treatment time and voltage gradient of the ACP had significant effects on theinactivation of *Alternaria alternata* spores and functional quality characteristics. This study is financially supported by Ege University Scientific Research Projects Coordination (Project No. FBG-2021-23013).

Keywords: Atmospheric cold plasma, tomato, exergy, antioxidant activity, quality.

Investigation of the antifungal activity of postbiotics

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ABSTRACT

Many different methods are used in the food industry to preserve food and the most common of these are chemical preservatives. However, today, due to the increasing concerns of consumers and the carcinogenic effects caused by chemicals, the need to research natural preservatives has emerged. Postbiotics are soluble materials (products or metabolic by-products) either secreted by Lactic acid bacteria (LAB) or released after bacterial lysis. Some of these materials including bacteriocins, organic acids, enzymes etc. offer antimicrobial activity. In this study, the antifungal effect of postbiotics obtained from three LAB (*Lactobacillus acidophilus, Lactobacillus plantarum, Lactobacillus rhamnosus*) against *Penicillium brevicompactum* mould isolated from muffin cake was investigated. Postbiotics were prepared in de Man, Rogosa and Sharpe medium in two stages from lyophilized granules at 37 ± 1°C (5% CO_2 atmosphere) for 24 hours and the bacterial suspensions were centrifuged at 4000g for 10 min and the supernatants were removed and freeze-dried. The freeze-dried extracts were dissolved in a sufficient amount of sterile water. After that, they were sterilized by filtration and mixed with Potato dextrose agar (PDA). The final concentrations of extracts in the medium were fixed at 40 mg/ml. The spore suspension was inoculated into the center of the agar plates and the diameter of the formed colonies was measured after 7 days of incubation at 25°C. Inhibition rates were calculated for postbiotics of *L. plantarum, L. acidophilus, L. rhamnosus* relative to negative control as 48.07% ± 5,65^{a}, 30.26% ± 3.45^{b}, and 3.06% ± 0.31^{c}, respectively. According to these results, it was concluded that the postbiotic produced by *L. plantarum* is the most effective one against *P. brevicompactum*. In this study, it was determined that postbiotics have antifungal activity and gave an idea that they can be used as an alternative to chemical preservatives in industrial bakery products.

Keywords: Antifungal, Bakery Products, Postbiotic, Preservative

Effect of dual-modification by heat-moisture treatment and octenyl succinic anhydride (OSA) on physicochemical and emulsion properties of arrowroot (*Maranta Arundinaceae* L.) starch

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ABSTRACT

Native arrowroot starch has some drawbacks that can limit its application in the food sector. These limitations can be overcome by modifying arrowroot starch using various methods to improve its functional properties and expand its application. In this study, arrowroot starch was modified using several methods, either single or dual modification methods, namely a) heat-moisture treatment (HMT), b) octenyl-succinic anhydride (OSA), c) HMT - OSA, and d) OSA and HMT. The results show that there was no damage to the surface of starch granules after modification using OSA, whereas slight damage occurred on the surface of the starch granules after modification using HMT, either single or dual modification (HMT, HMT-OSA, and OSA-HMT). All modification methods did not alter the crystallinity pattern but decreased the relative crystallinity of native arrowroot starch. Both dual modifications significantly decreased the degree of order and degree of double helices of native arrowroot starch. HMT either single or dual modification increased the pasting temperature and setback viscosity (easy to retrograde), and conversely, decreased peak and breakdown viscosities indicating the HMT treatment can improve the thermal stability of native arrowroot starch. The emulsifying activity (EA) of the OSA-treated starch, either single or dual modifications has higher EA than native and HMT starch, especially for OSA-HMT. This showed that the dual modification with OSA can increase the hydrophobicity of starch.

Keywords: arrowroot starch, heat-moisture treatment, octenyl-succinic anhydride, physicochemical, emulsion properties

Low-Intensity ultrasonics as a tool to control the quality of meat alternatives in-real-time during processing.

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ABSTRACT

Plant-based meat alternatives (e.g., extruded meat analogues such as patties and chunks) have gained global interest as healthy and sustainable substitutes of animal meat. The characteristic textural features as well as protein quality of such foods depend on ingredient properties, extrusion process conditions and protein alignment in the extruder die. Accordingly, development of desired textural and nutritional profiles in meat alternatives is a complex process that needs to be fully understood for optimized product properties. To tackle this challenge, low-intensity ultrasonics was utilized to monitor extrudates made from a blend of plant proteins at different moisture contents (50-70%) during manufacturing. The ultrasonic properties (phase velocity and attenuation) of the extrudates were assessed using an air-coupled ultrasonic system (200–600 kHz) placed at the die exit. Texture measurements were performed to acquire information on the level of protein texturization, which is a good signature of the formation of meat-muscle-like fibers in an extrudate. The in-vitro protein digestibility corrected amino acid score of extrudates was measured to determine the protein nutritional quality. The relationships between texture, nutritional profile and ultrasonic properties were modelled. Low-intensity ultrasound showed great potential as a non-destructive tool to monitor the quality of meat analogues in-real-time during processing.

Nutritional content and health benefits of orange and purple carrot-based smoothies enriched with sour cherry and apple juices during the 3- and 6-month storage periods

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ABSTRACT

Smoothies provide daily intake rates of fruit and vegetables and can be a form of second breakfast or afternoon snack. However, the stability of smoothies during the shelf-life is an issue for the beverage industry. Therefore, in the present study, orange and purple carrot-based purees with sour cherry and apple juices were used to prepare smoothies to explore physicochemical, bioactive compounds and biological activities during the 3&6-month storage periods to suggest the best product for the beverage industry. According to the study results, the highest viscosity, osmolality, anthocyanin, phenolic acid, and polymeric procyanidin contents; antioxidant activities; lipase and acetylcholinesterase (AChE) enzyme inhibition activities were observed in sour cherry juice-purple carrot puree included smoothies (SCJ-PC) immediately after production, during the 3- and 6-month storage periods. Lipase inhibition activities of SCJ-PC, during the 3-month storage period decreased, but during the 6-month storage, the inhibition activities increased. The reverse trend was observed for the AChE activities of SCJ-PC. In other words, during the 3-month storage period, the AChE inhibition of SCJ-PC increased but during the 6-month storage period, the inhibition of AChE decreased. Therefore, the study promotes more purple carrots as an ingredient in novel food products due to their high bioactive compounds and boosted immune system functions.

Keywords: AChE, BuChE, lipase, fruit juice

Development of Mediterranean Cereal Foods (Bread and Bulgur) With High Beta-Glucan Content

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ABSTRACT

The excessive consumption of fast foods, rich in sugar and fat, and low in essential nutrients is the main risk factor for the incidence of non-communicable diseases such as cardiovascular disorders, obesity, and diabetes. In 2010, UNESCO recognized the Mediterranean Diet as an Intangible Cultural Heritage of Humanity. A healthy and balanced diet combines well with the Traditional Mediterranean Diet which is characterized by cereal products (bread, pasta), a high intake of vegetables and fruits, a high intake of unsaturated lipids (particularly olive oil), the presence of plant proteins from legumes and moderate intake of fish and meat. MEDWHEALTH project will redesign a selection of typical Mediterranean Foods improving nutritional quality by using innovative raw materials. Med-Foods with higher b-glucan and protein contents and a lower glycemic index will be developed in the course of the project.

At this stage of the project, bulgur was produced from the high b-glucan hull-less barley cultivar Chifaa and its properties were compared with the bulgurs produced from a hull-less Turkish barley cultivar Yalin and a durum wheat cultivar Kiziltan. The moisture, protein, ash, and β-glucan contents of bulgur samples were determined. The protein contents of the bulgur samples prepared from Chifaa, Yalin, and Kiziltan were 10.55%, 13.40%, and 9.03% while the ash contents were 1.76%, 1.58%, and 1.33%, respectively. The β-glucan contents of the bulgur samples prepared from Chifaa, Yalin, and Kiziltan were 7.03 g/100g, 5.08 g/100g, and 0.56 g/100g. The cooking properties (cooking time, weight and volume increase during cooking, and total organic matter analysis) of bulgur samples were also evaluated.

Breads were also produced by supplementing wheat flour with barley flour at 15, 30, 45, and 60% supplementation levels. The flour obtained from a strong bread wheat cultivar (Tosunbey) was used to encounter the weakening effect of barley flour on rheological and baking properties. Farinograph properties of barley flour-supplemented dough samples were determined. Water absorption values ranged from 58.80 to 68.50% as barley flour content increased. The firmness values of barley flour-supplemented bread samples were between 601.72 and 2737.75 gram-force. The crust color values of barley flour-supplemented bread samples were also evaluated. L* values ranged from 49.81 to 65.24, a* values from 15.75 to 7.79, and b* values from 31.38 to 26.82 as barley flour content increased.

The diet in Mediterranean countries either includes around 200/day of bread or a roughly equivalent or in some cases a higher amount of other cereal products (couscous, bulgur, pasta, and/or cereal soups). Hence there is a good chance of consuming 3 g/day of b-glucans if barley is included in the diet of Mediterranean people in different forms.

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Keywords: barley, bread, bulgur, β-glucan

Synthetic hexaploid wheats: phenolic acid composition and antioxidant capacity

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ABSTRACT

Tetraploid durum wheat (*Triticum turgidum*, AABB) and diploid wild goat grass (*Aegilops tauschii* Coss., DD) are hybridized to produce synthetic hexaploid wheat (SHW, AABBDD) to bridge the gene-transfer from goat grass and durum wheat to hexaploid bread wheat (*Triticum aestivum*). More than 1000 accessions have been generated until now which is a rare instance of successful breeding using wild relatives of wheat. In this study, 21 synthetic hexaploid wheat samples were analyzed and compared for phenolic content (The Folin-Ciocalteu method), phenolic compositions, and antioxidant activity (DPPH, ABTS, and CUPRAC).

Bound, free, and total phenolic contents (TPCs) of the wheat samples were determined as 145.38-258.55 mg GAE/100g wheat, 188.19-369.38 mg GAE/100g wheat, and 333.58-576.93 mg GAE/100g wheat, respectively. Phenolic compositions were detected by the HPLC system. Gallic acid was found in the highest concentrations in free fractions, whereas gallic, p-coumaric acid and chlorogenic acids were generally found in the highest concentrations in bound fractions of the synthetic hexaploid wheat samples. The antioxidant activities (AA%) of the wheat samples were evaluated by the DPPH assay. AA% in the free extracts of the synthetic red wheat samples ranged from 33.0 to 40.5% and AA% values of bound extracts of the synthetic hexaploid wheat samples varied between 34.4 and 50.6%. ABTS and CUPRAC analyses were also used to measure antioxidant activities. The ABTS values of the synthesized hexaploid wheat samples' free and bound extracts and total ABTS values ranged from 27.31 to 123.18 mg TE/100g, 61.65 to 263.23 mg TE/100g, and 93.94 to 308.07 mg TE/100g, respectively. The CUPRAC values of the free and bound extracts and total CUPRAC values of synthetic hexaploid wheat samples were between 25.78 and 160.94 mg TE/100g, 75.35 and 308.13 mg TE/100g, and 107.51 and 364.79 mg TE/100g, respectively.

This study revealed that synthetic hexaploid wheat samples are valuable resources for breeding programs for developing new wheat varieties with higher concentrations and better compositions of health-beneficial phytochemicals. The samples w1 (Ukr.-Od. 1530.94/ *Ae. squarrosa* (629)), w18 (Ukr.-Od. 1530.94/ *Ae. squarrosa* (1027)), and w20 (Ukr.-Od. 1530.94/ *Ae. squarrosa* (392)) can be used as a genetic resource in breeding programs to enhance the nutritional quality of wheat.

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Keywords: synthetic hexaploid wheat, free phenolic, bound phenolic, gallic acid

The effect of biomaterials coating on sensory properties of potatoes during storage

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ABSTRACT

Under the current circumstances, there are many changes in the quality and sensory properties of food products. So, this work focused on potato due to it is the fourth most important food crop in the world after rice, wheat, and maize and the second drop after wheat in Jordan. we improved these characteristics of these products by renewable raw materials derived from agro-food (whey protein concentrate) waste as an edible coating on the surface of potatoes that the consumer can eat as a part of the whole product During 120 days of storage at different conditions storage. Chitosan, whey protein, and coconut oil (lipid) have been used. Along with uncoated (control) potato bulbs, the coated tubers were kept. They evaluated various quality characteristics such as shrinkage, wrinkles, visual appearance, and decay percentage. According to the findings, potatoes that had been coated had better results compared to control by delay shrinkage, wrinkles, decay percentage, and color changes.

Keywords: edible coating, chitosan, potato, quality

An alternative biological control method against *aspergillus carbonarius* growth

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ABSTRACT

Ochratoxin A (OTA) produced by *Aspergillus* spp. (*Aspergillus ochraceus, Aspergillus carbonarius, Aspergillus niger*) and *Penicillium* spp. (*P. verrucosum, P. nordicum, and P. viridicatum*) is one of the most significant mycotoxins of global concern for human and animal health due to its involvement in a wide range of toxicological consequences, such as kidney toxicity, mutagenicity, teratogenicity, and immunotoxicity. This study aimed to examine the effect of *Metschnikowia pulcherrima* isolated from grape leaves as a biocontrol agent against *A. carbonarius*, the OTA producer, by *in vitro* studies. The antagonistic activity of yeast against *A. carbonarius* was investigated using agar plate inhibition and dual culture method. Fungal growth was evaluated with the dual culture method on Malt Extract Agar (MEA) at 25 ºC and inhibition rates were observed for *Metschnikowia pulcherrima* 57D1AN as 71.43 ± 1.75. While the *A. carbonarius* used in the study shows optimum growth at 30°C and 0.97 aw, it produces OTA at 20 °C and 0.97 - 0.99 a_w. Therefore, agar inhibition assay by disk diffusion was conducted at 20, 25 and 30°C and 0.97 - 0.99 a_w. There were no growth observed on all tested temperatures and water activity values. This study suggests that, the application of antagonistic yeasts may be an alternative approach to control *A. carbonarius* growth and its OTA production with a more safe, feasible and green strategy.

Keywords: Antagonistic yeast, *Aspergillus carbonarius*, Biocontrol, Ochratoxin A

Green walnut extract: a novel ingredient for enhancing bee products

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ABSTRACT

This study introduces a new Bee feed product, the Green Walnut Bee Product, to address the challenges faced by Bees due to global warming. The depletion of natural food sources and the spread of diseases have had a negative impact on Bee populations, impacting agriculture and food security. In response to the growing demand for healthy foods, the study aimed to develop an innovative, healthy Bee product that supports Bee conservation. The Green Walnut Bee Product is prepared by fermenting green walnut extract with normal sugar and placing it in Beehives. The Bees readily consumed the feed, and after digestion, the product produced by the Bees was evaluated. The study found that the Green Walnut Bee Product had significant health benefits and could be utilized to produce high-quality, healthy Bee products. The technology has the potential to improve Bee health, contribute to sustainable agriculture, and provide a valuable source of nutrition for humans. The Green Walnut Bee Product offers a sustainable alternative to chemical-based products and has potential health benefits, including antioxidant, antimicrobial, and Blood sugar-reducing properties in Humans. The study highlights the importance of Bee conservation and the need to address the challenges faced by Bees. The Green Walnut Bee Product offers a promising solution to the problems faced by Bees due to global warming, while also providing a new segment of healthy Bee products for human consumption. The technology can be scaled up for commercial production, providing a new source of income for Beekeepers, promoting Bee conservation, and contributing to the local economy.

Keywords: Green walnut Extract, Bee Feed, Bee Product, Innovation, Bee conservation.

Nutritional value assessment of *adansonia digitata* leaves in sudan.

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ABSTRACT

Adansonia digitata is an African tree that has been known as a unique plant species with high health-promoting substances. The baobab tree (*Adansonia digitata*) is widely used in Africa for different purposes including, medicinal, food, and fruity beverages. Baobab leaves, seed oil, and fruit pulp are considered as most valuable foodstuff. This study aims to investigate the nutritional value of baobab leaves. The study area has covered six different ecological zones in Sudan and the laboratory work has been done in the laboratory of the Institute of food science, University of Debrecen, and an inductively coupled plasma emission spectrometer (ICP) is used for elemental analysis. The mineral composition of baobab leaves has been examined (and the result shows significant differences among means with the high nutritional value of subjected substance and ($p<0.05$). The highest and lowest concentration (Mg/100g) of (Ca, Na, Cu, and Fe) were shown in each zone (BRT 30713 and KPT 20920), (KWT 86.54, and BPT, 66.40), (BWT 10.540, and BRT 5.794), (KPT 198.9, and BPT, 117.8) These results showed varied compositions in terms of trees leaves' organ characteristics. Therefore, further studies in the physical and chemical characteristics of raw materials of baobab products are needed to provide essential information for food engineering and unit operations systems and predict the behavior of innovative baobab products.

Keywords: Adansonia digitate, Baobab, Innovation, Nutritional value.

Changes of biochemical compositions of blueberry fruit during shelf life

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ABSTRACT

Effects of preharvest hexanal spray treatment of Star, Beloxi, Legacy, Blueribbon blueberry varieties biochemical compositions were determined during shelf life. For this purpose Star, Beloxi, Legacy, Blueribbon blueberry varieties were treated with hexanal spray applications which were, doses (0%, 0.02%) prior to one day before harvest sprayed then harvested fruits were stored at 2°C and RH %90 conditions. Effects of hexanal spray applications of fruit physical and chemical composition were analyzed. Differences among treatments were identified and among treatments (0.02% hexanal and control) and cultivar and shelf life on anthocyanin, total phenol content and free radical scavenging capacity, sugar content of blueberry fruit storage at 2°C and RH %90 conditions at initial time, 6 and 12 days during shelf life. Results showed that, hexanal application positively effected the biochemical composition of anthocyanin, total phenol content and free radical scavenging capacity, sugar content and weight loss and firmness during shelf life. The hexanal application were effected anthocyanin, total phenol content and free radical scavenging capacity amount of blueberry varieties. As a result, hexanal application has important effects on biochemical compositions of blueberry varieties during shelf life.

Keywords: blueberry, shelf life, hexanal spray, quality

Effects of preharvest hexanal treatment on chemical compositions of rasberry fruit during storage

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ABSTRACT

Changes in anthocyanin, total phenol content and free radical scavenging capacity compositions and weight loss, brix, sucrose, glucose, xylose, fructose and total sugar content of raspberry were determined in Diamond Jubile, Jade rasberry varieties releated to pre harvest hexanal application during shelf life. Diamond Jubile, Jade rasberry varieties were treated with hexanal spray applications (0%, 0.02%) preharvest and stored at 2°C and RH %90 conditions. Effects of hexanal spray applications of rasberry fruit chemical compositions were analyzed in a spectrophotometer and determined by HPLC techniques. Differences among treatments were identified in chemical compositions at four days intervals during 10 days of storage life. Results showed that, hexanal application effected the amount of anthocyanin, total phenol content and free radical scavenging capacity, weight loss, brix, sucrose, glucose, xylose, fructose and total sugar composition during storage life. Finally, preharvest application were determined that signifant effects on chemical composition of rasberry during storage.

Keywords: rasberry, shelf life, hexanal spray, quality

Edible insects: tendency or necessity for functional foods

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ABSTRACT

Eating insects has been a widespread habit in many cultures for many years. Edible insects represent an innovative food source with many advantages that will help the problem of protein and energy shortages created by the rapid growth of the world population. Using insects as food can increase the economy and help protect the environment and the human survival. Their nutritional value is excellent, since according to many studies insects have high protein content, high concentrations of various essential amino acids, a well-balanced fatty acid profile, with a high content of monounsaturated, polyunsaturated fatty acids and many minerals, trace elements, and vitamins. However, there are several risks in the use of edible insects, which need to be researched more extensively. The entomophagy has many advantages. However, the consumption of insects is associated with several potential risks, such as allergens. For this reason, in this research, the existence of allergenic proteins in the larvae of the *Tenebrio molitor* beetle and the influence of their diet on their allergenicity, were studied according to EU regulation 1169/2011.

Pectin and gelatine based nanocomposite biodegradable films containing sweetgum bark extract

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ABSTRACT

The present research focused on developing active food packaging utilizing sweet gum bark extracts through zein nanoparticles. D-optimal combined design is used to investigate the relationships between mixture variables (carbohydrate and protein ratios) and process variables (glycerol and zein nanoparticles ratios) to obtain an optimal response. Active nanocomposite films were produced by incorporating zein nanoparticles loaded with bioactive extract into pectin and gelatine-based films. Mechanical properties, water vapor permeability, opacity and colour values, nanocomposite films' antimicrobial activity, and volatile compounds in optimum film samples were detected. Permeability properties against water vapor (0.28-0.96 g.mm/m2.h.kPa) were moderate regarding food application. Pectin and gelatine-based nanocomposite films incorporated with sweet gum bark extracts showed antimicrobial activity against foodborne pathogens, including *Escherichia coli*, *Listeria monocytogenes* and Staphylococcus aureus. The activity was highest against *Listeria monocytogenes* with 1.13 log cfu/g inhibition. L-limonene was the main volatile with 29.68 % area in the developed nanocomposite films.

Keywords: nanocomposite film, sweetgum bark extract, active food packaging, gelatin, pectin

1 INTRODUCTION

In recent years, the production rate has been increasing in agriculture and the food industry to provide the necessary amount of food for the increasing population. Accordingly, one of the issues that are gaining importance worldwide is the evaluation of waste. In this respect, phenolic substances in natural agricultural wastes are attractive due to their broad bioactivity spectrum and low cost (Panzella et al., 2020). Among the food packaging systems, active packaging systems can be formed with various active compounds showing antioxidant and antimicrobial properties, such as natural plant extracts, essential oils, organic acids, etc. (Ramos et al., 2013). The latest trends focus on using active compounds derived from natural and effective alternative sources. Plants are well known as the sources of many biologically active molecules possessing antimicrobial properties (Negi, 2012).

The Anatolian sweetgum tree draws attention mainly because of the sweetgum oil balm, a functional product (Arslan & Şahin, 2016). There are some studies in which the properties of leaves, bark and oil of sweetgum are determined and used as a natural preservative in herbal treatments. The antifungal effect of methanol extracts from sweetgum leaves collected from Muğla province against microorganisms that cause fungal diseases in cucumber and apple was determined. It was emphasized that sweetgum leaf extracts could be an alternative natural control method instead of synthetic pesticides (Onaran, 2018). The ethanol extract obtained from sweetgum leaves has high antioxidant activity (96.55%), the total phenolic content is an average of 333.14 ± 7.96 mg GAE/g extract, and the primary phenolic acids in its composition are protocatechin acid, (-) epicatechin. and gallic acid (Saraç and Şen, 2014). Moreover, it was found that the extracts of sweetgum leaves obtained with different solvents (acetone, ethanol and methanol) have antimicrobial effects on a wide variety of microorganisms, including *Bacillus subtilis*, *Escherichia coli*, *Salmonella Typhimurium*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Candida albicans*, *Yersinia enterocolitica* (Okmen et al., 2014). Sweetgum bark is a remarkable natural waste that reveals its functional properties through further studies and evaluating the obtained products with alternative and innovative methods.

Carbohydrate-based films are formed due to the remodelling of the polymer chain by evaporation of the solvent, forming hydrophilic and hydrogen bonds in a film matrix. Since these films are composed of hydrophilic polymers, so they have a low moisture barrier. Proteins provide advantages with networking and elasticity properties in obtaining biodegradable packaging (Rhim & Perry, 2007). Films obtained using different protein and carbohydrate compositions show better barrier properties and improve their mechanical properties. Due to the hydrophilic characteristics, pectin-based biodegradable films have a poor barrier to water and weak mechanical properties. For this reason, composite structures are formed with different carbohydrates and proteins to improve their mechanical properties, especially their barrier properties against water. Gelatin is a kind of protein which has been commonly used for active food packaging (Shen et al., 2021). Gelatin-based biodegradable films are primarily developed as carriers of antioxidant or antimicrobial compounds. The mechanical and barrier properties of gelatin-based active films enriched with green tea extract were improved, and the addition of green tea extract showed a significant increase in the films' total phenolic content and antioxidant activity (Wu et al., 2013). In another study, gelatin-based active films with antioxidant properties were developed using five different extracts: green tea extract, grape seed extract, grape seed polyphenols, ginger extract, and ginkgo leaf extract. Adding ginkgo leaf extract provides the highest antioxidant activity in gelatin-based films and improves the film's light and water vapor permeability properties (Li et al., 2014).

The food industries are currently utilizing nanotechnology for food preservation using food packaging. Nanodispersions are used to encapsulate, protect and transfer bioactive components such as antimicrobials and antioxidants because of many advantages such as better stability, increased bioactivity and good dispersity (Prakash et al., 2018). In this study, an extract of sweetgum bark wastes was used as a source of active compounds for developing pectin and gelatine-based active nanocomposite films. The extract of sweetgum bark wastes was encapsulated in zein nanoparticles and incorporated into a pectin and gelatine composite film. Optimization of nanocomposite film was performed based on the films' mechanical properties, water vapor permeability and opacity values.

2 MATERIALS AND METHODS

2.1 Production of zein nanoparticles

Sodium caseinate was dissolved in water at 1% (w/v) concentration, and zein was dissolved in 80% (v/v) ethanol at 7.50 mg/mL concentration. Sweetgum bark extract was added to zein solution at 3.50 mg/mL. Zein-sodium caseinate nanoparticles were prepared based on the high-speed homogenization method. In the high-speed homogenization method, zein solution was dropwise to an equal amount of sodium caseinate solution under the rotational speed of 8250 rpm for 4.3 minutes.

2.2 Production of nanocomposite films

The amount of gelatin to form the total dry matter (%10) was transferred into distilled water and was kept in a water bath at 70°C for 10 minutes. The pectin solution was mixed with a homogenizer at 4000 rpm for 5 minutes. The prepared solutions were placed in an ultrasonic bath and degassed for 10 minutes. Then, the carbohydrate solution was transferred to the protein solution, and glycerol was added to the homogeneous composite film solution. The film solutions were poured into aluminium cups and dried in a tray dryer at 45°C at an air speed of 1 m/s until they reached a constant weight. According to the D-optimal combined trial design, 28 different films were produced. Carbohydrate (X1) and protein (X2) ratios were mixing variables, while the process variables were glycerol ratio (X3) and zein nanoparticles ratio (X4).

Table 1. D-optimal composite design used in the production of nanocomposite films.

Experiment Number	Carbohydrate ratios	Protein ratios	Glycerol (% dry based)	Zein Nanoparticules (% dry based)
1	12.50	87.50	20.00	5.00
2	0.00	100.00	10.00	5.00
3	25.00	75.00	10.00	10.00
4	25.00	75.00	20.00	10.00
5	0.00	100.00	10.00	10.00
6	25.00	75.00	10.00	5.00
7	12.50	87.50	20.00	10.00
8	0.00	100.00	20.00	5.00
9	12.50	87.50	10.00	5.00
10	12.50	87.50	10.00	10.00
11	0.00	100.00	20.00	10.00
12	25.00	75.00	20.00	5.00
13	12.50	87.50	10.00	7.50
14	0.00	100.00	15.00	5.00
15	25.00	75.00	15.00	7.50
16	12.50	87.50	15.00	7.50
17	0.00	100.00	10.00	7.50
18	25.00	75.00	20.00	7.50
19	6.25	93.75	20.00	7.50
20	6.25	93.75	15.00	10.00
21	18.75	81.25	15.00	5.00
22	25.00	75.00	15.00	10.00
23	6.25	93.75	12.50	6.25
24	25.00	75.00	10.00	10.00
25	0.00	100.00	20.00	5.00
26	0.00	100.00	10.00	10.00
27	25.00	75.00	10.00	5.00
28	0.00	100.00	10.00	5.00

2.3 Film Thickness

The thickness of the films was measured using a digital micrometre (Mitutoyo, 99MAB039M1-Model MDC-SX, Japan) with an accuracy of 0.001 mm. Thickness measurements were randomly made from 6 different points for each film, and average thickness values were used in the calculations.

2.4 Water Vapor Permeability

The water vapor permeability of the films was determined gravimetrically using the modified standard ASTM E96-80 (1981) method. For permeability measurement, 3.5 cm diameter discs were cut from the films. Film discs were placed on 50ml specific cups containing 2g silica gel. The water vapor permeability was calculated as g.mm/m2.h.kPa by taking the weights of the containers which were kept at 25 °C for 24 hours under the ambient conditions of 75% relative humidity provided with saturated NaCl solution and the film layer exposed to 0% relative humidity.

2.5 Color and Opacity

The color properties of the films were determined based on the CIELAB color scale using a colorimeter (Colorimeter, PCE-CSM-5). Color parameters including L (lightness/brightness), a* (green/red) and b* (blue/yellow) were evaluated. Color properties in films are expressed as total color difference (ΔE*).

Opacity value of films were measured according to method performed by Dou et al. (2018). Each film to be tested was cut in dimensions of 10×45 mm in accordance with the dimensions of the spectrophotometer cuvette. Absorbance values were measured at 600nm wavelength in a UV-Visible (Schimadzu, UV-1601, Japan) spectrophotometer. The opacity values of the films were calculated using equation 1 given below.

$$\text{Opacity} = (A_{600}) / X \quad (1)$$

A_{600}: absorbance value at 600nm, X: Film thickness (mm)

2.6 Mechanical Properties

Tensile stress (MPa) and elongation at break (%) values of composite films were measured with TA.XT texture analyzer using ASTM D882-88 (1989) standard method. The films were cut in 6cm x 1.5cm dimensions and placed between the two jaws of the probe and tested at the speed of 0.50 mm/min until the film broke. Measurements were made by taking four different sections from the film sample.

2.7 Antimicrobial Activity

The antimicrobial effect of the film samples was tested against the microorganisms *Listeria monocytogenes*, *Escherichia coli* and Staphylococcus aureus in a liquid medium using the ASTM E2149-10 (2014) standard method. Test microorganisms *Escherichia coli*, and Staphylococcus aureus were activated in Nutrient Broth (NB) medium for 24 hours at 37 °C and *Listeria monocytogenes* at 25 °C for 24 hours. Cells were counted in the range of 106-107 CFU/ml. Active nanocomposite film discs were aseptically transferred into test tubes containing 10 ml of culture medium and incubated at 37 °C for 24 hours. At the end of the incubation, serial dilutions were made in peptone water solutions and then inoculated into Plate Count Agar (PCA) medium by pouring method. *E.coli* and S.aureus inoculated petri dishes were incubated at 37 °C, L.monocytogenes inoculated at 25 °C for 24 hours and bacterial colonies were counted at the end of the incubation. Inoculation was also made from the culture medium without a film disc for each microorganism. Results are expressed as inhibition in log cfu/g based on the number of colonies (log cfu/g) in culture medium with and without film.

2.8 Analysis of Volatile Compounds

Volatile compounds in the active nanocomposite films were determined using the solid-phase micro-extraction technique (SPME) and gas chromatography-mass spectroscopy (GC-MS) (Shimadzu, GC-2010 Plus, Kyoto, Japan) (D'auria et al., 2004). For this purpose, 1 g of film sample was transferred to 20 mL vials, sealed and kept at 45°C for 10 minutes. Then, PDMS/DVB fiber (65 µm, Supelco, Bellefonte PA/USA) was placed in the headspace of the samples and waited for 30 minutes at 45°C to absorb volatile components into the fiber. After solid phase microextraction, the fiber absorbing the volatile components was transferred to the GC-MS sampling port. Under the following conditions, the volatile component analysis was performed using a capillary column (Restek Rxi-5ms, USA; 30 mx 0.25 mmID x 0.25µm). GC-MS analysis conditions: injection block temperature: 250 ºC; desorption time: 3 min, splitless mode; GC-column temperature program: 1 min at 50ºC; 3 ºC/min increase to 200ºC, 8 ºC/min increase to 250ºC and hold at 250ºC for 5 min; MS-scan mode: 35-450 m/z; ionization energy: 70 eV; interface temperature: 250 ºC; mobile phase: helium (44.4 cm/s). The volatile compounds were identified using libraries registered on GC-MS (Wiley9N11,lib. and Nist11,lib.).

3 RESULTS AND DISCUSSION

3.1. Water Vapor Permeability

The water vapor permeability values of nanocomposite films containing zein nanoparticles loaded with sweetgum bark extract varied between 0.28 and 0.96 g.mm/m2.h.kPa. Each component in the film formulation affects the water vapor permeability of films. However, the amount of nanoparticle solution is lower than other film components, and its effect on water vapour permeability is more limited than other components. The model perturbation graphs indicated that a sharp linear increase in water vapour permeability is observed depending on the increase in the glycerol ratio. An increase in the ratio of zein nanoparticles loaded with sweetgum extract also showed a moderate increase in the water vapor permeability of the films.

3.2. Color and Opacity

A low opacity value is expressed as a high transmittance indicator for the food packaging films. The desired opacity property may differ depending on the type of food to which the packaging films or coatings will be applied. For example, high light transmittance is a prerequisite for fruit packaging materials (Thakur et al., 2016). The colour differences of the sweetgum extract-based nanocomposite films were determined as a minimum of 6.10 and a maximum of 28.24. Opacity values were found in the range of 0.27 to 1.55. ANOVA results showed that mixture and process variables significantly affect the opacity value in sweetgum-based films.

Compared to the studies in the literature, the colour values of the nanocomposite films were found to be slightly higher. Dou et al. (2018) found colour differences ranging from 1.04 to 3.73 and opacity values ranging from 1.33 to 1.43 in alginate and gelatin-based composite films. It can be concluded from the perturbation graphs that pectin and zein nanoparticles in the formulation of composite films are effective on the colour properties of nanocomposite films.

3.3. Mechanical properties

While the tensile strength of the sweetgum bark extract-based nanocomposite films ranged from 5.77 MPa to 39.59 MPa, the elongation at break (%) values were determined between 84.66% and 160.61%. The tensile strength of the films was affected by the mixture and process variables at a statistically significant ($p<0.05$) level. The minimum tensile stress was obtained under conditions where the protein ratio of the mixture variables was 75%, the carbohydrate ratio was 25%, and the process variables were 20% glycerol and 10% zein nanoparticles. On the other hand, the maximum tensile stress was obtained in the film experiment where the protein ratio was 100%, the carbohydrate ratio was 0%, the glycerol ratio was 10% and the zein nanoparticle ratio was 5%. The increase in the protein ratio and the corresponding decrease in the carbohydrate ratio increased the tensile stress of the nanocomposite films.

The elongation percentage of the films was also affected by mixture and process variables at a statistically significant ($p<0.05$) level. The glycerol ratio affected the elongation percentage of the films statistically significantly and the percentage of elongation of the films increased with the increase in the glycerol ratio. It is observed that the elongation values of the films were maximum in conditions where the protein ratio is maximum and the carbohydrate ratio is minimum in the film composition when the process variables are constant at a definite point.

The mechanical properties of biocomposite films are affected by many factors, such as biopolymers in the film composition, plasticizing agents, drying conditions of the films and functional additives. Liu et al. (2015) examined the effect of adding sodium acetate into gelatin films and drying them at different temperatures while the tensile strengths of the films dried at 35°C ranged from 31.1 to 71.5 MPa. Their elongation percentages were between 2.5 and 34.6 %, tensile strengths of the films dried at 25°C ranged from 47.3 to 71.5 MPa and elongation percentages varied between 5.1% and 30.2%. Regardless of the temperature, the elongation percentages of gelatin-based films are quite low due to the rigid structure of gelatin after drying. For this reason, it is advantageous to form gelatin-based films with another biopolymer, such as pectin, in a composite structure.

3.4. Antimicrobial activity

The antimicrobial effects of active bionanocomposite films were investigated against the microorganisms *Listeria monocytogenes*, *Escherichia coli* and *Staphylococcus aureus*. The results are given in Table 2. In nanocomposite films containing zein nanoparticles loaded with sweetgum bark extract, antimicrobial activity was detected against *E. coli*, ranging from 0.11 to 0.91 log cfu/g. The antimicrobial activities of active nanocomposite films against *L. monocytogenes* were found to be close to their antimicrobial activities against *Escherichia coli*. The antimicrobial activity of films ranged from 0.22 to 1.13 log cfu/g against *L. monocytogenes*, while it was between 0.27 and 0.64 log cfu/g against *Staphylococcus aureus*. The highest antimicrobial activity was determined against *L. monocytogenes* in one of the films formulated with a maximum zein nanoparticles ratio (%10). In contrast, the lower antimicrobial activity was observed against *Staphylococcus aureus.*

3.5. Volatile compounds

Volatile compounds and area percentages determined in nanocomposite films containing zein nanoparticles loaded with sweetgum bark extract are given in Table 3. For the sweetgum bark extract-based nanocomposite films, the sum of the peak areas of the volatile compounds identified on the chromatogram constituted 99.9% of the total peak area. The main volatile components in films containing sweetgum bark extract are L-limonene, palmitate <isopropyl->; hexadecanoic acid <1-methylethyl-> ester, dihydro jasmonate <methyl->; cyclopentaneacetic acid <3-oxo-, 2-pentyl-, methyl-> ester, 1-(4-Isopropylphenyl)-2-methyl propyl acetate, 8-heptadecene, 1-chloro- and hedione; cyclopentaneacetic acid is defined as <3-oxo-, 2-pentyl-, methyl-> ester. L-limonene is the main volatile component with a higher area percentage than others in active nanocomposite films. In the study by Erdoğdu (2022), acetophenone (23.2%), cinnamyl alcohol (18.5%), benzene, 1,3-diisocyanatomethyl- (11.1%), α-methylstyrene (10.3%) and cinnamaldehyde (10.2% were the main determined volatile compounds. α-methyl styrene (1.51%) is one of the volatile compounds found in sweetgum bark extract itself and in nanocomposite films, including the extract developed in this research.

Table 2. Properties of active nanocomposite films including zein nanoparticles loaded with sweetgum bark extract.

Experiment Number	WVP (g.mm/m^2.h.kPa)	Color difference (ΔE)	Opacity	Tensile Strength (MPa)	Elongation at break (%)	*Escherichia coli* inhibition	*Listeria monocytogenes* inhibition	*Staphylococcus aureus* inhibition
1	0.72 ± 0.01	18.07±1.01	0.73 ± 0.03	7.45 ± 0.18	131.22±0.40	0.15 ± 0.01	0.55 ± 0.03	0.43 ± 0.04
2	0.39 ± 0.01	21.64±0.34	0.35 ± 0.01	39.59 ± 1.33	84.66±0.14	0.11 ± 0.03	0.69 ± 0.02	0.40 ± 0.02
3	0.38 ± 0.01	14.65±0.76	1.36 ± 0.04	22.3 ± 1.61	100.47±1.15	0.21 ± 0.01	1.13 ± 0.12	0.55 ± 0.03
4	0.96 ± 0.06	6.10±0.05	0.86 ± 0.01	5.77 ± 0.11	96.72±0.12	0.14 ± 0.01	0.99 ± 0.11	0.64 ± 0.07
5	0.42 ± 0.02	28.24±0.29	0.42 ± 0.01	33.43 ± 1.88	96.06±0.69	0.18 ± 0.01	0.80 ± 0.04	0.59 ± 0.04
6	0.37 ± 0.01	18.13±0.16	1.41 ± 0.07	34.74 ± 1.14	93.15±1.30	0.28 ± 0.02	0.58 ± 0.05	0.55 ± 0.05
7	0.74 ± 0.01	8.25±0.01	0.81 ± 0.01	10.52 ± 0.84	132.14±0.09	0.34 ± 0.03	0.63 ± 0.04	0.45 ± 0.01
8	0.53 ± 0.02	21.95±0.13	0.42 ± 0.01	23.35 ± 0.83	123.18±0.04	0.25 ± 0.03	0.44 ± 0.01	0.31 ± 0.02
9	0.37 ± 0.03	21.44±0.07	0.90 ± 0.01	34.7 ± 0.21	89.39±1.83	0.78 ± 0.09	0.48 ± 0.03	0.27 ± 0.02
10	0.44 ± 0.01	7.04±0.02	1.05 ± 0.02	23.7 ± 0.25	96.31±1.36	0.42 ± 0.04	0.73 ± 0.06	0.29 ± 0.12
11	0.50 ± 0.02	29.35±0.13	0.53 ± 0.03	10.32 ± 0.06	160.61±0.59	0.33 ± 0.05	0.79 ± 0.09	0.54 ± 0.03
12	0.78 ± 0.01	21.23±0.04	1.55 ± 0.03	6.62 ± 0.04	122.74±1.64	0.83 ± 0.03	0.42 ± 0.02	0.49 ± 0.02
13	0.42 ± 0.01	7.85±0.02	1.16 ± 0.01	27.01 ± 0.36	86.32±0.41	0.66 ± 0.04	0.60 ± 0.07	0.57 ± 0.04
14	0.46 ± 0.01	24.26±0.23	0.27 ± 0.01	25.78 ± 0.14	111.06±0.5	0.64 ± 0.01	0.44 ± 0.05	0.42 ± 0.01
15	0.42 ± 0.01	22.89±0.08	1.38 ± 0.01	7.67 ± 0.29	89.97±1.33	0.75 ± 0.03	0.32 ± 0.06	0.53 ± 0.08
16	0.46 ± 0.01	8.34±0.04	1.10 ± 0.01	16.18 ± 0.65	121.5±0.53	0.46 ± 0.02	0.41 ± 0.04	0.52 ± 0.02
17	0.43 ± 0.01	13.94±0.23	0.59 ± 0.01	39.17 ± 1.61	94.5±0.65	0.80 ± 0.03	0.39 ± 0.03	0.50 ± 0.03
18	0.78 ± 0.01	15.53±0.19	1.09 ± 0.02	10.5 ± 1.54	106.37±0.38	1.03 ± 0.06	0.49 ± 0.02	0.57 ± 0.04
19	0.64 ± 0.03	11.82±0.01	0.89 ± 0.01	12.2 ± 0.24	146.24±0.29	0.23 ± 0.02	0.40 ± 0.04	0.59 ± 0.04
20	0.49 ± 0.01	22.52±0.09	0.74 ± 0.01	11.88 ± 1.13	138.8±0.01	0.79 ± 0.02	0.56 ± 0.04	0.63 ± 0.03
21	0.50 ± 0.01	20.78±0.12	1.22 ± 0.01	12.6 ± 0.56	116.89±1.15	0.58 ± 0.02	0.34 ± 0.03	0.42 ± 0.01
22	0.44 ± 0.02	12.08±0.05	1.26 ± 0.02	9.43 ± 0.92	95.13±0.40	0.91 ± 0.04	0.44 ± 0.02	0.58 ± 0.05
23	0.33 ± 0.05	22.72±0.06	0.63 ± 0.01	26.38 ± 0.44	105.21±0.24	0.71 ± 0.03	0.32 ± 0.04	0.47 ± 0.07
24	0.28 ± 0.01	8.17±0.01	1.41 ± 0.01	27.08 ± 0.16	106.96±0.99	0.61 ± 0.07	0.27 ± 0.04	0.51 ± 0.03
25	0.59 ± 0.02	6.81±0.07	0.35 ± 0.01	16.55 ± 0.26	129.89±0.25	0.40 ± 0.01	0.32 ± 0.07	0.36 ± 0.02
26	0.44 ± 0.04	24.59±0.07	0.6 ± 0.01	26.79 ± 0.26	104.61±0.54	0.37 ± 0.01	0.36 ± 0.04	0.48 ± 0.01
27	0.32 ± 0.01	7.11±0.01	1.37 ± 0.01	36.37 ± 0.01	106.11±0.20	0.30 ± 0.01	0.24 ± 0.03	0.29 ± 0.04
28	0.32 ± 0.01	7.11±0.01	0.29 ± 0.01	38.54 ± 1.57	84.75±0.31	0.27 ± 0.01	0.22 ± 0.03	0.27 ± 0.03

Table 3. Identified volatile compounds in nanocomposite films containing zein nanoparticles loaded with sweetgum bark extract.

Peak Number	Volatile compound	Area (%)
1	l-Limonene	29.68
2	Neryl acetone	4.44
3	Benzenepropanal <4-(1,1-dimethylethyl)-, alpha-methyl-	3.15
4	Dihydrojasmonate <methyl->; Cyclopentaneacetic acid <3-oxo-, 2-pentyl-, methyl-> ester	10.16
5	1-(4-Isopropylphenyl)-2-Methylpropyl Acetate	8.76
6	8-Heptadecene, 1-chloro-	5.31
7	Hedione; Cyclopentaneacetic acid <3-oxo-, 2-pentyl-, methyl-> ester	5.70
8	4-Propionyloxytridecane	4.28
9	.ALPHA. HEXYLCINNAMIC ALDEHYDE	1.84
10	Caprylate <octyl->; Octanoic acid <octyl-> ester	1.85
11	7-Acetyl-6-ethyl-1,1,4,4-tetramethyltetralin	3.77
12	N-Benzylformamide	3.08
13	Palmitate <isopropyl->; Hexadecanoic acid <1-methylethyl-> ester	14.54
14	.alpha.-Methylstyrene	1.51
15	Cinnamyl cinnamate	1.92

4 CONCLUSION

In conclusion, active packaging films are attractive solutions to maintain the quality of foods. These films can be designed with nanocomposite formulations to protect the activity coming from active components. The presented study evaluated sweetgum bark extract as a natural source of active compounds and incorporated nanoparticles loaded with extract into biodegradable film materials for active packaging film solutions. The developed nanocomposite films containing zein nanoparticles loaded with sweetgum bark extract are eco-friendly active packaging films showing antimicrobial activities. According to water vapor permeability and mechanical properties, selected film formulations are able for food applications in the future.

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Production of shalgam juice and its' functional properties

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ABSTRACT

Shalgam juice is a fermented beverage which has red colored, cloudy and acidic features containing bulgur flour, sourdough, water and salt and is obtained with addition of sliced purple carrot (Daucus carota) and turnip to fermented liquid (Brassica rapa) after lactic acid fermentation that lasts about 2-4 weeks. The characteristic red color of shalgam juices arises from purple carrot that is the main ingredient of the beverage. The positive effects of shalgam juices on health come from functional components such as anthocyanins and phenolic acids which are in high amount in purple carrot.

Shalgam juice has functional properties and microbiologically safe, nutritional food. Due to the protective properties of lactic acid formed by fermentation, it is known that lactic acid fermentation has been used in the preservation and stabilization of fruits and vegetables throughout history. It is known that turnip juice has positive effects on the digestive system due to the lactic acid it contains and, like most fermented products, it has an appetizing feature. Lactic acid gives turnip juice a sour taste as well as facilitating digestion, refreshing, regulating the pH of the digestive system and it also gives the body features that allow the body to benefit more from some minerals. Since the development of pathogenic microorganisms is prevented in products produced by lactic acid fermentation, the foods in question are considered safe products for health. In this review article, production technology and functional properties of shalgam juice are discussed.

Keywords: Functional properties, lactic acid, Shalgam juice, traditional fermented beverage.

Impact of oat-drink residue flour on the white bread's dough properties and baking characteristics

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ABSTRACT

The aim of this work is to study the structure-function relationship of dry fractionated oat drink residue (DFOR) as a technological ingredient, using bread as a model system. Dried oat drink residue was mechanically fractionated into three different particle sizes DFOR1:<150µm, DFOR2:150-224µm, and DFOR3:224<300µm and blended with bread wheat flour at 10% and 20% substitution levels for bread making. The blended flours and bread samples were assessed for their dough mixing and bread technological characteristics. Results from Mixolab, Rapid Visco Analyser, and gel strength showed that, inclusion of DFOR exhibited a higher ($p<0.05$) water absorption values compared with control. Similarly, both final viscosity and setback values were significantly increased ($p<0.05$) particularly at 10% substitution levels, whereas 10% mixtures showed the lowest breakdown peak viscosity, indicating a reduction of starch content. Gel strength results showed that the addition of the oat-drink residue flour yields a softer gel texture, which translated to a softer crumb texture in the final baked products. Analysis of bread samples showed that oat-drink residue flour supplementation resulted in increased bread loaf volume at 10% inclusion levels, while bread samples showed volume reduction as the oat-drink residue flour concentration increased. Colour analysis revealed that the lightness (L*) of the crust and crumb of the bread samples decreased from DFOR1 to DFOR3, as oat-drink residue flour supplementation increased from 10 to 20%. Incorporating oat drink residue has the potential to be utilized in bread preparation in the context of sustainability in food production. Results highlight the potential for incorporating fractionated oat drink residue in modifying the properties of wheat flour for bread-making applications and improving the sustainability of the oat drink production process.

The value of the waste products of date fruit (*phoenix dactylifera*)

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ABSTRACT

Date fruits produce dry sweet fruits with different morphological and biochemical characteristics and many of the prime varieties are consumed as such while less-desired varieties are processed mainly into date paste and date syrup. The date seeds and pomace, which are waste by-products from this processing, are not valorized.

The date fruit pomace is a rich source of dietary fiber and polyphenols. The dietary fiber is mainly insoluble fiber consisting of cellulose, hemicellulose, and lignin. The phenolic fraction is dominated by melanin constituted by epicatechin-based oligomeric pro-anthocyanidins (5-15%). The pro-anthocyanidins are known for several bioactivities including anti-inflammatory and anticarcinogenic effects. The date fruit pomace can be used in a wide range of food and pharmaceutical products. Date fruit seeds represent around 10% of the fruit weight and contain insoluble carbohydrates, lipids, proteins, and ash. Date seeds are less explored mainly due to their very hard structure caused by cellulose and mannan polysaccharides. They are also reported to have hormonal activities that needs to be evaluated before their inclusion in functional foods is suggested. Nevertheless, they can be included in several non-food applications.

Encapsulation of aqueous hibiscus sabdariffa extract in high internal phase emulsions stabilized by soy protein isolate

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ABSTRACT

Unlike common emulsions, High Internal Phase Emulsions (HIPEs) are more resistant to different factors such as phase separation, temperature and pH. Thanks to at least 74% internal phase volume, it allows encapsulation of bioactive compounds in high volumes. Traditionally, HIPEs are stabilized with common surfactants. However, many studies show that the consumption of surfactants has adverse effects on the gastrointestinal tract, intestinal microbiota and cell toxicity. For this reason, studies on the use of plant-based proteins as stabilizers or emulsifiers have increased in recent years. At this point, soy protein isolate (SPI) has an important potential to be used as a Pickering stabilizer with its solid structure.The aim of this study is to encapsulate aqueous *Hibiscus Sabdariffa* extract, rich in anthocyanins and phenolics, in HIPEs stabilized with soy protein isolate and soy lecithin, to evaluate the emulsion stability and the changes during *in vitro* gastrointestinal digestion. In this study, HIPEs consisting of aqueous Hibiscus extract (80%) and corn oil (20%) phases stabilized with different combinations of 2%, 4% and 6% lecithin and 2%, 4% and 6% SPI gels were prepared. As the used SPI gel concentration increased, the stability of the emulsion increased. During *in vitro* digestion, as the SPI gel concentration increased, the total anthocyanin content (TAC) of the emulsions decreased by approximately 50% compared to the aqueous Hibiscus extract. While the TAC did not show a statistically significant change in the gastric phase, it presented approximately 30% increase during intestinal phase.

Keywords: Hibiscus Sabdariffa, High Internal Phase Emulsion, *In vitro* digestion, Soy Protein Isolate

Investigating the antimicrobial susceptibility of raw chicken *campylobacter* isolates to erythromycin and benzalkonium chloride

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ABSTRACT

Campylobacteriosis is the leading cause of foodborne infections worldwide and chicken meat is the main reservoir of *Campylobacter* spp. bacteria which are responsible for the disease. In this work, six *Campylobacter* strains isolated from raw chicken meat (three *C. jejuni* and three *C. coli*) and having different rep-PCR profiles, were tested for their sensitivity to one macrolide antibiotic (erythromycin, ERY) and one general purpose biocide (benzalkonium chloride, BAC). The *C. jejuni* strain ATCC 33291 was used as reference. The minimum inhibitory and minimum bactericidal concentrations (MICs and MBCs, respectively) of each compound were determined against each strain, through the broth microdilution (BMD) and agar spot assays, respectively. The BMD assay was performed in Muller-Hinton broth, either supplemented with 5% laked horse blood (MH-HB) or without (MH). Resazurin was also employed as metabolic indicator when MH was the growth medium used (to facilitate MIC determination). The results showed that ERY was effective against five of the six meat isolates and the reference strain, with MIC values ranging from 0.25 to 4 μg/mL in MH and from 0.25 to 2 μg/mL in MH-HB. The MBCs for that compound ranged from 0.5 to 4 μg/mL in both nutrient media. Remarkably, there was also one *C. jejuni* meat isolate with high-level resistance to ERY when this was grown in MH (MIC greater than 128 μg/mL), whereas the MIC in MH-HB was much lower and detected at 16 μg/mL (with MBC at 64 μg/mL). Regarding BAC, the MICs against all *Campylobacter* strains were greater in MH-HB (2 – 8 μg/mL) than in MH (1 – 4 μg/mL). The same was also true for the MBCs (4 – 32 μg/mL and 1 – 4 μg/mL, respectively). Overall, the obtained results reveal the great influence of the nutrient medium on the efficacy of two well-known antimicrobial agents against *Campylobacter* bacteria. Studies are in progress to determine whether there is any correlation between the observed phenotypic resistance and genomic content.

Keywords: *Campylobacter* spp., antimicrobial resistance, erythromycin, benzalkonium chloride

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Probiotic viability during the shelf life of a novel greek sheep traditional yogurt and following subsequent *in vitro* digestion

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ABSTRACT

Probiotic viability in yogurt during shelf life and subsequent survival after passage through the gastrointestinal tract, is crucial in determining the health benefits of the product. Besides nutritional value, the enhancement of yogurt's sensory characteristics contributes to the consumer's acceptance. The aim of this study was to: a) obtain a new probiotic sheep yogurt with upgraded quality, b) monitor the population of lactic acid bacteria (LAB) species throughout the product's shelf life, and c) evaluate the survival of probiotic bacteria in a static *in vitro* digestion model (SIVDM). To do this, yogurt was manufactured the traditional way by fermenting pasteurized milk with either only the commercial starter culture (*Lactobacillus delbrueckii* subsp. *bulgaricus (LB)*, *Streptococcus thermophilus (ST)*; YF-L812 Chr Hansen) (CY), or together with the potential probiotic strain 708 of *Lacticaseibacillus rhamnosus (LR)*, isolated from raw sheep milk (PY). The survival of all three LAB species was monitored throughout the product shelf life (incubation at 4 ^{o}C for 20 d), and also following exposure to SIVDM. At each sampling day (5, 11, 16 and 20 d after production), both yogurt samples (CY, PY) were also evaluated for their main sensory characteristics (appearance, odor, taste, texture, and overall acceptance) by 15 panelists. The population of probiotic LR remained stable during the shelf life (and above 10^8 CFU/g). Similar good was also the survival of ST (with counts always above 10^9 CFU/g). On the contrary, the initial population of LB (10^6 CFU/g) was not detected from the 11^{th} d and afterwards ($<10^2$ CFU/g). Yogurt starters were not detected following SIVDM, whereas LR (in PY) presented a reduction of about only one log. Sensory analysis did not reveal differences between the two yogurt types during the shelf life. To sum, the novel yogurt had good sensory attributes and was able to deliver to consumer a high amount of potentially probiotic cells.

Keywords: probiotic yogurt, digestion, bacterial survival, *Lacticaseibacillus rhamnosus*

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Antioxidant and metal chelating activities of dandelion (*taraxacum officinale* (g.h. weber ex wiggers) leaves

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ABSTRACT
Taraxacum officinale (G.H. Weber ex Wiggers) (Asteraceae), commonly known as dandelion, is a perennial plant found worldwide, considered as a weed by most gardeners; has been used for centuries as a natural medicine. This plant is produced for medicinal purposes and food, either grown from wild sources or cultivated. Young leaves of *Taraxacum officinale* are also used as a food in salads and vegetable dishes, due to its nutritional value; containing high concentrations of fiber, minerals, vitamins and essential fatty acids. Aside its culinary uses, dandelion is well known in traditional medicine for its therapeutic properties. Roots, leaves, and flowers are commonly used for homemade remedies, being useful in the treatment of a variety of complaints, such as diabetes type 2, spleen and liver complaints, as well diuretic. *Taraxacum* contains several pharmacologically active compounds like, flavonoids, and it is capable of preventing damage of cells during oxidative stress. In the present study, antioxidant activity (inhibition of lipid peroxidation, iron chelation, DPPH)-scavenging) were investigated. The extracts of *Taraxacum* leaves have exhibited strong antioxidant activity in the lipid peroxidation assay. 100 g of methanol extracts exhibited 89.8% inhibition of peroxidation in linoleic acid system respectively and greater than the same dose of a-tocopherol (52.6 %). As a result, *Taraxacum* leaves showed strong antioxidant and antiradical scavenging and metal chealating activities which may contribute to the interpretation of the pharmacological and medicinal effects of *Taraxacum officinale*.; representing a promising source for the prevention and treatment of health conditions. Although protective action of *Taraxacum officinale* against, oxidative stress is well reported in the scientific literatüre; further research is needed for validating the medicinal properties of this plant as a health remedy.

Keywords: *Taraxacum officinale*, Antioxidant, Lipid Peroxidation, Free Radical Scavenging, Metal Chelating

Free radical scavenging and metal chelating activities of *beta vulgaris* subsp. *maritima* (L.) arcang

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ABSTRACT
An ancient plant, sea beet (*Beta vulgaris* subsp. *maritima* (L.)) is the wild ancestor of common vegetables like beetroots and Swiss chard, belonging to Chenopodiaceae family, known for centuries for its pharmacological activities, and nutritional properties. It is Native to Europe and the Mediterranean, and also known as sea spinach, wild beet and wild spinach. It can be found by salt marshes, coastal, sandy areas near the sea like beaches and sea walls. It is resistant against many pathogenes, nematodes and insects. Moreover, *B. maritima* is highly tolerant to drought, heat and, salinity. It contains phenolic compounds, well-known as antioxidants, which may play a significant role in the human diet due to their potential health beneficial effects. *B. maritima* contain phenolics that inhibited free radical production. In the present study, the antioxidant activity (inhibition of lipid peroxidation, iron chelation, DPPH)-scavenging) has been investigated. Furthermore, pigment contents, total contents of phenolics and flavonoids are evaluated. The extracts of *B. maritima* leaves have exhibited strong antioxidant activity in the lipid peroxidation assay. 100 g of methanol extracts exhibited 92.4% inhibition of peroxidation in linoleic acid system respectively and greater than the same dose of a-tocopherol (78.8%). These extracts also been detected to have effective free radical scavenging, and metal chelating activities. As a result, *B. maritima* extracts showed strong antioxidant and antiradical scavenging and metal chealating activities which may contribute to medicinal effects of this plant. Although the antioxidant profiles of *B. maritima* roots and leaves are well-known, metal chelating potential of this plant is not evaluated efficiently. Thus, the aim of this study was to determine the pigment and phenolic contents of *B. maritima* plant and to clarify the role of phenolics in metal chelating processes.

Keywords: *Beta vulgaris* subsp. *maritima* (L.), Phenolics, Free Radical Scavenging, Metal Chelating

Light spectral modulation of phenolic synthesis in *ocimum basilicum* L.

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ABSTRACT

Dietary phenolic substances are a valuable pool of health maintaining compounds and increasing their synthesis in raw materials will lead to increased economic value. Light spectrum modulation was used during growth of Ocimum basilicum L microgreens and phenolic compound synthesis was qualitatively and quantitatively assessed. Far red, blue and UV light regimes led to modest (+2%) to significant (+20.9%) increases in phenolic synthesis, compared to white light spectrum. While leaf surface, plant mass and chlorophyll fluorescence were comparable under various light regimes antioxidant activity of basil extracts increased with up to 15% under modified light spectrum. With similar costs for LED illumination as in the case of regular, white light, raw food materials such as basil can have increased economical value which will translate to subsequent resulting products, using modulated spectrum.

Polycaprolactone cojugation reduces siloxane toxicity towards crop and microbial species

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ABSTRACT

The siloxane/polycaprolactone hybrid material combines properties of two different polymers with potential in various biomedical applications. The obtained biodegradable polymer by ring-opening polymerization of ε-caprolactone (CL) using aminopropyl-polydimethylsiloxane (APDMS) oligomer and their effects towards on the growth and development of tomato plants (Lycopersicum esculentum) were investigated. The siloxane/polycaprolactone hybrid material obtained was characterized by FTIR spectroscopy, NMR spectroscopy, energy dis-persion spectroscopy (EDX) analysis and the effects of this compound on the evolution of tomato plants (Lypercosium esculentum) were followed, but also on the biological stability by identifying some microorganisms developed on the surface, given its susceptibility to biodegradation. Experimental results in the light of the parameters studied to demonstrate the initiation of biodegradation of the tested samples. By initiating the process of biodegradation, products are released into the soil, which influences the growth and development of plants, analyzed parameters have slightly lower values compared to the reference sample (in the vessel without siloxane material, the plants have developed better, than if they were introduced into the soil alongside the tested matrices), but being at the acceptable level. The development of microorganisms, in particular Fusidium viride and Penicillium brevicompactum, first identified on hybrid polymeric materials based on polycaprolactone, also confirms that these products do not have a major disturbing effect on soil composition and plant evolution and show acceptable susceptibility to the environment. Moreover, due to the superior performances compared to the individual materials and through the possibilities of modeling the properties according to the field of use, the researched hybrid can be a solution for the future, without negatively influencing the bioremediation of the soil.

A simple electrochemical method for nickel detection in vegetables and fruits

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ABSTRACT

The evaluation of the quality and safety of raw and processed food is crucial when there are people allergic to different compounds in the food matrix, such as metal ions. Allergy to nickel is one of the problems faced by more and more people, so the development of methods for the rapid identification of these ions is important in preventing some symptoms immediately after consuming some foods with nickel content. Rapid methods should be available to anyone with this type of allergy to identify the content of nickel ions before consuming the food. The method expected by hypersensitized people could be one of the types of electrochemical biosensors, portable and disposable. In this study, such a method was developed by using protein A-agarose as a biological element and silver and bismuth screen-printed electrodes. Electrochemical measurements were carried out by cyclic voltammetry and a bipotentiostat was used to study the electrochemical behavior of the nickel-protein complex formed. Determinations of the nickel content were made with this biosensor on products such as pears, plums, tomatoes, and cucumbers and were compared with the results of the nickel content of the same products but by Atomic Absorption Spectrometer (AAS) which is the reference method. The results showed a similarity of data obtained from AAS with the biosensor method, thus characteristics of the performance were also analyzed.

Keywords: electrochemical biosensors, food safety, nickel allergy

Designing of texture-modified fruit juices by adding different hydrocolloids

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ABSTRACT
The aim of this study is to develop texture-modified fruit juices (orange, apple, and peach juices) that had been prepared with hydrocolloids, namely guar gum (GG), xanthan gum (XG), and starch (S) at different concentrations. Thickened liquids classified at three National Dysphagia Diet levels were stored at 4°C for three months. Physicochemical analyzes (total phenol determination, antioxidant capacity, pH, titration acidity, color, viscosity, syringe flow test, fork drip test) of the fruit juices were determined before and after storage. The sensorial perceived differences and flow behavior between ascending concentrations for all studied thickeners for orange juice samples were investigated at the end of the three-month storage. Ten panelists rated the viscosity and swallowing difficulty for each orange juice sample on a 9-point hedonic scale. Subsequently, they also evaluated overall acceptability and five texture attributes including appearance, aroma, taste, color, texture using a 5-point hedonic scale to gain information about sensory taste and mouthfeel. The results showed that physicochemical properties of samples were significantly changed with fruit and hydrocolloid type and storage period. It was found that results obtained syringe flow test carried out according to the International Dysphagia Diet Standardization Initiative framework were not compatible to the National Dysphagia Diet categories used for preparing texture-modified liquids. Sensory evaluation showed that orange juices containing starch were more acceptable compared to other samples. Compared to starch and guar gum, fruit juices containing xanthan gum showed higher values of viscosity and swallowing difficulty and were less preferred by the panelists. In order to develop safe standard recipes for dysphagic patients, all preparation and storage conditions must be considered affecting TM foods and fluids.

Keywords: dysphagia, hydrocolloid, texture food, viscosity

The effects of using chia and flaxseed as egg replacements on the quality of the cakes

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ABSTRACT
In this study, it was aimed to develop a cake suitable for the consumption of individuals who do not consume eggs for various reasons by using chia and flaxseed gel as egg substitutes in a cake formulation, and to examine the effects of using chia and flaxseed gel as egg substitute on cake quality. Wheat flour, egg, milk, oil, sugar, water, baking powder and different proportions of chia seed and flaxseed gels were used in the production of cake samples. Chia (50%, 100%) and flaxseed (50%, 100%) gels were added to the formulation separately, replacing eggs. Physical analyzes of the cakes were made in parallel at 2 and 24 hours after baking. The specific volume of the samples prepared within the scope of the study was calculated and dry matter content, total ash content and sensory analyzes were made. The obtained data were analyzed with the SPSS statistical program. As part of the sensory analysis, ten panelists rated each cake sample on a 9-point hedonic scale. Panelists evaluated the products for color, appearance, texture, taste, aroma and overall acceptability. There was no statistically significant difference between the cake samples in the specific volume measurements made at the 2nd and 24th hours of the cake samples. In dry matter analysis, statistically significant difference was found between the samples at the 2nd and 24th hours. While there was no statistical significance in the ash analysis at the 2nd hour, a statistically significant difference was found among the samples at the 24th hour. In sensory evaluation, it was determined that the control group and cakes containing 50% chia and flaxseed gels were more acceptable than cakes containing 100% chia and flaxseed gels. The sensory properties of cakes made with less popular gels containing 100% Chia and flaxseed need to be further developed.

Keywords: Sensorial properties**,** chia seed, egg replacement, flaxseed

Biochemical composition and health effects of *Phaeodactylum tricornutum*

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ABSTRACT

Microalgae can be grown in bioreactors without farmland and are considered an alternative food source for human nutrition because they are rich in valuable compounds such as proteins and fatty acids. *Phaeodactylum tricornutum* is an aquatic, unicellular, eukaryotic microalgae containing high amounts of protein, carotenoids, fucoxanthin (Fx), and eicosapentaenoic acid (EPA), an omega-3 polyunsaturated fatty acid (n-3 PUFA). In this study, it was aimed to comprehensively investigate the protein and EPA content, amino acid content, fucoxanthin content, aroma compounds, and compounds effective on human health of *Phaeodactylum tricornutum*. The total protein content and EPA content of *Phaeodactylum tricornutum* were determined as 26.55% and 13.90%, respectively. The amount of fucoxanthin, which is not a provitamin but a carotenoid, was found to be 174.6 mg/100g. Aspartic acid, glutamic acid, glycine, arginine, and alanine amino acids were determined by HPLC-PDA in *Phaeodactylum tricornutum*, and it was observed that the most dominant amino acid was glutamic acid. The aroma compounds were analyzed by HS-SPME-GC/MS, and acetic acid was determined as the most dominant aroma compound. It is known that fucoxanthin and EPA in the structure of *Phaeodactylum tricornutum* have biological activities such as antioxidant, anti-inflammatory, anti-obesity, and anti-cancer.

Keywords: *Amino acid, Aroma, Fucoxanthin, Phaeodactylum tricornutum*

Bioactive Composition and Antioxidant Activity of *Spirulina platensis*

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ABSTRACT

Microalgae, especially *Spirulina platensis*, have a rich nutritional composition, including carbohydrates, lipids, proteins, vitamins, minerals, and bioactive compounds essential for basic human nutrition. It is known to have anticancer, antioxidant, anti-inflammatory, neuroprotective, hepatoprotective, and hypocholesterolemic properties because of the bioactive compounds it contains. This study aimed to comprehensively investigate the protein amount, amino acid content, total phenolic composition, and antioxidant capacity of *Spirulina platensis* grown commercially (C-SP) and under laboratory conditions (LC-SP). It was determined that the total protein content of *Spirulina platensis* grown under laboratory conditions (55.12%) was higher than the commercial sample (43.93%). The main amino acids of LC-SP are glutamic acid and alanine, while in the C-SP, they are arginine and aspartic acid. The main amino acids of LC-SP are glutamic acid and alanine, while in the C-SP-coded sample, they are arginine and aspartic acid. The total amount of amino acids in LC-SP (230.6 mg/100g) is higher than in C-SP (115.8 mg/100g). It was determined that the LC-SP has approximately 2-3 times higher antioxidant capacity and total phenolic substance (TPC) content than the C-SP coded sample. *Spirulina platensis* has a substantial supply of antioxidants and valuable secondary metabolites, besides its potential commercial application and medicinal properties. This study enlightens the fact that *Spirulina platensis* can be consumed as an alternative food supplement rich in protein, amino acids, and antioxidants.

Keywords: Antioxidant activity, Amino acids, Protein, Spirulina platensis

1. INTRODUCTION

Cyanobacteria, also known as blue-green algae (*Cyanophyta*), are photosynthetic prokaryotic organisms classified as gram-negative. They thrive in diverse habitats, ranging from aquatic environments to terrestrial settings, including salt lakes, deserts, polar regions, and hot springs (Borowitzka, 2018).

Spirulina platensis (*S. platensis*) is a microalgae cultivated globally for its nutritional value and the production of phycocyanin, a blue pigment used in cosmetics and food. Historical records suggest that the Aztecs consumed "*Arthrospira platensis*," a nutrient-rich food source, particularly abundant in vitamin B12 and protein, harvested from Lake Texcoco in Mexico (Ciferri, 1983; Fox, 1996). Similarly, local communities around Lake Chad have been harvesting *S. platensis* for years, using it as a nutritional supplement known as "dihe" (Siva Kiran et al., 2015).

Among *Spirulina* species, *Spirulina fusiformis* (*Arthrospira fusiformis*), *Spirulina platensis* (*Arthrospira platensis*), and *Spirulina maxima* (*Arthrospira maxima*) have undergone extensive research due to their high nutritional and potential medicinal properties (Deng & Chow, 2010). The World Health Organization (WHO) has endorsed *S. platensis* as a valuable natural source of secondary metabolites with economic and therapeutic potential, commonly used in human and animal food supplements (Hadizadeh et al., 2019). Both NASA and the European Space Agency have recommended it as a primary food source for long-term space missions due to its exceptional nutritional value (Deng & Chow, 2010). Its cell wall, composed of 86% digestible polysaccharides, is easily digestible by humans (Li & Qi, 1997).

S. platensis comprises approximately 15% carbohydrates, 70% protein, 7% minerals, 5% fats, and 3% moisture. Unlike other plant-based proteins, it provides a balanced mix of essential and non-essential amino acids (Colla et al., 2007; Saranraj & Sivasakthi, 2014).

S. platensis and its components have demonstrated numerous health benefits, serving as a complete protein source for various human health applications, from addressing malnutrition to providing antioxidant properties (Ravi et al., 2010). It contains active phytochemicals, including chlorophyll (green pigment), zeaxanthin (yellow pigment), xanthophylls (myxantophyll, zeaxanthin, cryptoxanthin, echinone), and carotenes (α-carotene, β-carotene, euglenanone, lutein). Additionally, it contains C-phycocyanin (blue pigment) and allophycocyanin, which are phycobiliproteins making up around 20% of its dry weight and holding significant economic value (El-Baz et al., 2013).

The functional and nutritional potential of *S. platensis* has garnered significant attention in recent years. This study aims to comprehensively investigate the protein content, amino acid composition, total phenolic content, and antioxidant capacity of commercially available *Spirulina platensis* and specimens grown under controlled laboratory conditions. Amino acid profiles was determined using HPLC-PDA, and antioxidant capacity was assessed using DPPH and ABTS methods.

2. MATERIAL AND METHOD

2. 1. Chemicals

Folin-Ciocalteu reagents were bought from Merck (Darmstadt, Germany), and Trolox and 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and amino acid standards were also procured from Sigma-Aldrich Chemical Co. (St. Louis, USA). All the solvents and chemicals utilized in this research were chromatographic and analytical grade.

2.2. Growth condition of *S. plantensis*

The commercial *Spirulina* samples were procured from local markets in powder form in Turkey. *S. platensis* culture was produced under controlled laboratory conditions at Adana Alparslan Türkeş Science and Technology University in Adana, Türkiye. The culture was conditioned at room temperature at 25 °C, and grown at a light intensity of 80 µmol photon $m^{-2}s^{-1}$ under laboratory conditions. Continuous illumination was applied and the light intensity was checked by a light meter (Licor, LI-250). Fluorescent lamps (Tekfen, TLD, 36 Watt) were utilized and the cultures placed on the shelves were ventilated by an aquarium air pump (3.5 L/min, 5 Watt). The trial culture groups were kept in six-liter flasks. Zarrouk medium (Aiba and Ogawa, 1977) was modified and used to produce *S. platensis* cultures.

2.3. Preparation of *S. plantensis* extract

In a 50 ml erlenmeyer flask, one gram of samples was weighed, 20 ml of water was put in, and the mixture was mixed at room temperature for one night in the dark. It was then kept in an ultrasonic water bath for 5 hours, and the mixture was centrifuged for 15 minutes at 4°C at 5.500 rpm. Then, 2 ml of the liquid was withdrawn and passed through a 0.45-size filter. The commercial *Spirulina* was named C-SP, while *S. platensis* grown under laboratory conditions is named LC-SP.

2.4. The total protein content analysis

Protein analysis is required to determine the amount of a specific protein in a mixture, the amount of non-protein hydrogen, and the nutritional value of nutrients. The basic principles of the method are the determination of nitrogen and peptide bonds. The total protein content was analyzed using the Kjeldahl method (AOAC, 2007; Cemerolu, 2014) in this study.

2.5. Amino acid profile by HPLC-PDA

The amino acid profile was determined based on the method explained by Zeng et al. (2015) with slight modifications. Dried samples of 0.5 g were hydrolyzed with 10 ml of 0.1 N HCL at 110°C for 24 hours under reflux. Then, 2 ml of the liquid was withdrawn and passed through a 0.45-size filter. A Shimadzu HPLC device (Prominence-i LC-2030C model) was used in the analyses. Samples derived with 3-MPA, OPA, and FMOAC were injected into the Agilent Eclipse Plus column C18 (3.5 µm, 4.6 x 100mm). 20 mM potassium phosphate buffer (45/40/15: acetonitrile/methanol/water) (pH 6.9) was used as the mobile phase. The amino acids were detected according to the retention times of the standards and the calculations were performed according to the peak areas at 262 nm for proline and at 338 nm for other amino acids.

2.6. Antioxidant activity and total phenolic content analyses

2.6.1. DPPH Method

This analysis was performed using 2,2-diphenyl-1-picrylhydrazyl (DPPH), which determined the sample's ability to inhibit free radicals using a UV-Vis spectrophotometer at 515 nm (BMG Labtech, Spectrostar Nano, Ortenberg, Germany) according to the method outlined by Brand-Williams et al. (1995). By mixing extracts with a DPPH solution, the color of the solution changed from purple to yellow based on the corresponding hydrazine. To determine the reducing ability of the antioxidants towards DPPH, the decrease in absorbance at 515 nm was monitored. Trolox concentrations ranging from 50 to 500 mM were used for the calibration, and the results were expressed as micromoles of Trolox equivalent (TE) per 100 g of dry weight (mM of TE/100 g of DW).

2.6.2. ABTS Method

In this method, 2, 2'-azino-bis 3-ethylbenzothiazoline-6-sulfonic acid (ABTS) was used based on the method of Saafi et al. (2009). Then, 7 mM ABTS was mixed with 2.45 mM potassium bisulfate and kept in the dark for 12–16 h and this solution was diluted with sodium acetate (pH 4.5) buffer to obtain an absorption value of 0.70 ± 0.01 at 734 nm in a spectrophotometer. Then, 2.98 mL of the prepared solution was mixed with 20 µL of sample extract and the absorbance was measured 10 min later in a UV Vis spectrophotometer (BMG Labtech, Spectrostar Nano, Ortenberg, Germany) at a wavelength of 734 nm. The absorbance values were calculated with the Trolox standard curve and the results were expressed in mM Trolox/100 g DW.

2.6.3 Total phenolic content analyses

TPC analysis was performed using the Folin–Ciocalteu reagent according to the method specified by Shahidi & Ambigaipalan (2015). Then, 200 µL of the extract/standard solution and 1.5 mL of Folin–Ciocalteu reagent (1:10) were added to the spectrophotometer cuvette. After five minutes, 1.5 mL of 6% sodium carbonate solution was added to the tubes and kept for 90 min at room temperature in the dark. The absorbance values were measured at 765 nm in a UV-Vis spectrophotometer. For the calibration curve, a 500 ppm gallic acid solution was prepared and the results were reported as mg/100 g DW.

2.7. Statistical Analysis

Statistical data analysis was conducted using SPSS 22.0 with One-way ANOVA (version 22, SPSS Inc., Chicago, IL, USA). Duncan's test measured the variations in the content levels of the results. Means with *p*-values below 0.05 are statistically significant.

3. RESULTS AND DISCUSSION

3.1. The total protein content of samples

Protein accounts for 60-70% of Spirulina's dry weight, depending on the source (Phang et al., 2000). Recently, there has been increasing relevance in gaining some peptides and biofunctional proteins from microalgae. Algal proteins are highly quality with extensive examination and nutritional studies (Becker, 2007; Samarakoon & Jeon, 2012).

There are studies on the effects of proteins and peptides obtained from micro-algae, such as antihypertensive, antioxidant, and anti-inflammatory (Samarakoon& Jeon, 2012; Raposo et al., 2013). The total protein content of S. platensis samples is given in Table 1. It was determined that the total protein content of LC-SP (55.12%) was higher than C-SP (43.93%) in this study ($p< 0.05$).

In a study, the amount of protein in Moroccan *Spirulina* was investigated, and it was stated that this strain contained a significant amount of protein (76.65%) (Seghiri et al., 2019). The nutritional value of *Spirulina* generated in the standard culture medium was found to have the highest total protein content at 52.95% (Madkour et al., 2012). In another study examining the impact of culture conditions on the chemical composition of *S. platensis* was determined that the total protein content was between 49.47 and 59.79% of dry weight (Marrez et al., 2014). Protein, peptides, and amino acids provide both nutritional and physiological benefits. Because of its protein-rich and amino acid profile, *S. platensis* could be used as a nutraceutical or functional food ingredient to prevent diseases and cell/tissue damage (Raposo et al., 2013). These findings support the hypothesis that *S. platensis* has a higher protein content than some plants and that the amount of protein varies depending on the growing conditions.

3.2. Amino acid profile

A total of 19 amino acids were determined in *S. platensis* samples (Table 1) and the amino acid composition of LC-SP includes arginine and aspartic acid, while alanine and isoleucine are the main amino acids in C-SP ($p<0.05$). LC-SP contains more amino acids (230.6 mg/100g) than C-SP (115.8 mg/100g). Glutamine and histidine were not detectable in both samples.

S. platensis is considered a complete protein source as it contains all essential amino acids. However, it has lower amounts of methionine, cysteine, and lysine compared to animal-based protein sources such as meat, eggs, and milk. Alanine is known to be available in some bacteria's cell walls and peptide antibiotics' structures. Other dominant compounds, aspartic and glutamic acid, are the main parts of the proteins and are known to have great importance for brain functions. Proline content was determined as 0.85 mg/100g and 3.48 mg/100g for C-SP and LC-SP, respectively.

Table 1: Total protein content and amino acid profile of *S. platensis* samples

Analyses	**C-SP**	**LC-SP**
Total protein (%)	43.93±0.23	55.12±0.21
Amino acids (mg/100 g)		
Aspartic acid	11.06±0.09^{a}	35.82±0.04^{b}
Glutamic acid	11.06±0^{b}	4.69±0.23^{a}
Asparagine	3.27±0.02^{a}	30.81±0.35^{b}
Serine	5.49±0.30^{b}	2.27±0.04^{a}
Glutamine	nd	nd
Histidine	nd	nd
Glycine	6.35±0.05^{a}	10.92±0.18^{b}
Threonine	6.94±0.40^{b}	5.16±0.28^{a}
Arginine	4.67±0.04^{a}	67.52±7.67^{b}
Alanine	13.65±0.71^{b}	1.25±0.18^{a}
Tyrosine	6.32±0.62^{b}	5.69±0.55^{a}
Cysteine	3.25±0.11^{a}	17.91±1.34b
Valine	6.38±0.31^{b}	5.63±0.26^{a}
Methionine	2.75±0.11^{a}	2.83±0.09^{a}
Tryptophan	7.41±0.34^{a}	21.21±0.05^{b}
Phenylalanine	2.50±0.04^{a}	7.77±0.95^{b}
Isoleucine	12.85±0.02^{b}	2.79±0.15^{a}
Leucine	10.96±0.97^{b}	4.26±0.46^{a}
Proline	0.85±0.08^{a}	3.48±0.01^{b}
Total	**115.8±0.20^{a}**	**230.06±2.09^{b}**

nd: Not determined

Different letters (a–b) on the same row indicate statistical differences ($p < 0.05$).

3.3. Antioxidant activity and total phenolic content

S. platensis is considered a rich source of nutritional phenolic and flavonoid compounds due to its higher production capacity than traditional plant-based sources. The easy participation of phenolic compounds in oxidation-reduction processes occurring in internal and exterior cells results in their antioxidant action (Atouia et al., 2005). It is known that phycocyanin, fucoxanthin, and phenolic compounds in the structure of *S. platensis* samples exhibit antioxidant activity. Table 2 shows the antioxidant capabilities of the samples evaluated in the current study.

Table 2: The antioxidant activity and TPC of *S. platensis* samples

Analyses	C-SP	LC-SP
DPPH (µmol Trolox /100g DW)	1.76±0.32[a]	5.71±0.05[b]
ABTS (µmol Trolox /100g DW)	159.93±5.78[a]	218.33±12.05[b]
TPC (mg/100 g DW)	2.38±0.66[a]	7.14±1.28[b]

Different letters (a–b) on the same row indicate statistical differences ($p < 0.05$).

It was determined that the LC-SP extract has approximately 2-3 times higher antioxidant capacity and total phenolic content (TPC) than the C-SP ($p<0.05$). The highest DPPH capacity was observed in the LC-SP with 5.71 µmol Trolox/100 g DW, while the lowest activity was detected in the C-SP (1.76 µmol Trolox/100 g). The highest ABTS capacity was determined as 218.33 µmol Trolox/100 g DW in the LC-SP coded sample (Table 2). The total phenolic content (TPC) of *S. platensis* samples is presented in Table 2. The highest TPC was observed in the LC-SP coded sample with 7.14 mg GA/100 g DW. In three previous studies, the TPC contents of the *S. platensis* samples were reported as 12.2 g/kg by Bolanho et al. (2014), 146 mg GA/100 g by Esquivel-Hernández et al. (2017), and 318–340 mg GA/100 g by Martelli et al. (2020). Elloumi et al. (2020) utilized different amounts of NaCl in an MDM medium to test the influence of salinity on the development and production of *Scenedesmus sp.* microalgae.

The use of antioxidant response mechanisms by microalgae can help prevent the effects of reactive oxygen species (ROS). The ROS and antioxidant response mechanisms vary depending on the microalgae species and are influenced by factors such as cell size, shape, density, growth stage, light, temperature, nutrients, and abiotic stress. Various parameters such as extraction conditions, also affect the amount of phenolic compounds present. Factors like time, temperature, and type of solvent can impact the quantity of phenolic compounds. Therefore, optimizing these extraction conditions is crucial for maximizing the yield of phenolic compounds (Ugya et al., 2020).

4. CONCLUSION

The effects of different growth conditions on the protein, amino acids, and antioxidant activities of *S. platensis* were investigated in this study. The LC-SP-coded sample's total protein and amino acid content was higher than C-SP. The main amino acids of the LC-SP sample are arginine and aspartic acid, while in the C-SP are alanine and isoleucine. The highest antioxidant capacity (AC) and total phenolic content (TPC) were determined in the LC-SP-coded sample. When the results were evaluated, it was shown that the differences in total protein, amino acid antioxidant activity, and TPC of *S. platensis* may vary depending on the growing conditions.

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Competition of interest

The authors declare that they have no competing financial interests or personal relationships that could affect the work reported in this article.

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A comparative study on phytochemical evaluation of *citrus auriantium* and *citrus paradisi* juices

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ABSTRACT

Citrus has been used all over the world for producing a variety of value-added and nutrionally improved food products, including juices, wines, jams, canned citrus and dried citrus. Citrus juices are consumed majorly because of their nutritional value and special flavor. They are significant source of bioactive components with nutritional and health-promoting metabolites, such as organic acids and phenolic compounds. Thanks to its chemical composition, it has a number of beneficial effects on health, including antioxidant, anti-inflammatory, antitumor and antimicrobial activities. In order to increase our knowledge on this subject, the characterization and quantification of *Citrus aurantium*, *Citrus paradisi*, Star ruby juices, (which are the species of *Citrus paradisi*), were analyzed using HPLC-DAD-MS. As a result of the analysis, 8 phenolic compounds were determined in all samples, while 12 phenolic compounds were detected in *Citrus paradisi* and its species Star ruby. The dominant phenolic compound was determined as naringin in *Citrus paradisi*, Star ruby and *Citrus aurantium* and their amounts were determined as 386.86±0.97 mg/L, 692.89±2.53 mg/L, 602.97±1.62 mg/L, respectively. Naringin has various biological and pharmacological properties such as protective effects against cardiovascular and neurodegenerative diseases, anti-carcinogenic, lipid-lowering, anti-apoptotic and anti-oxidant activities. While neohesperidin (559.1±3.53 mg/L) was found in high amounts in *Citrus aurantium*, gallic acid, quercetin 3-O-glucoside(ısoquercitrin), didymin and poncirin compounds could not be detected. In addition, high amounts of quercetin-3-O-rutinoside (337.79±1.79 mg/L) and hesperidin (59.39±0.12 mg/L) were found in *Citrus Aurantium*. Narirutin compound was dominant in Star ruby (178.47±2.66 mg/L), followed by *Citrus paradisi* (122.22±0.56 mg/L). This research has shown that citrus juices could be recommended in dietary habits as a potential source of phenolic compounds.

Keywords: citrus juice, HPLC-DAD-MS, phenolic compounds

Determination of differences in sulfur compound composition of fresh and black garlic samples

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Garlic (*Allium sativum L.)* is widely consumed worldwide as a vegetable, flavoring agent and herbal medicine. It is well known for its health benefits due to various bioactive compounds such as allicin, polysaccharides, vitamins, proteins, lipids, trace elements Se, flavonoids and 33 different organosulfur compounds and polyphenols. The organosulfur compounds of white garlic are altered during the black garlic production process. In this study, changes in the organosulfur content of white garlic and black garlic from Kahramanmaraş and Gaziantep were determined by liquid chromatography-electrospray ionization-tandem mass spectrometry (LC/ESI-MS/MS). Differences in organosulfur compounds of black garlic produced from white garlic were determined. Alliin, S-allyl-L-cysteine, y-glutamyl-S-allyl-L-cysteine, allicin (3R,5S)-5-methyl-1,4-thiazan-3-carboxylic acid, S-allyl-l-cysteine sulfoxide, S-(trans-1-propenyl)-l-cysteine sulfoxide were determined. S-alk(en)yl-l-cysteine content was determined to be increased in black garlic compared to fresh garlic.

Keywords: Organosulfur compounds, Allicin, Garlic, Black garlic

INTRODUCTION

For thousands of years, garlic has been widely used as a food and medicinal herb all throughout the world (Charron et al. 2016). Garlic is thought to have originated in Central Asia and moved from there to China, the Middle East, and the Mediterranean. Garlic is one of the oldest known horticultural herbs, with historical evidence indicating that it was employed by societies over 5000 years ago (USDA, 2006). Potasyum, fosfor çinko ve kükürt, selenyum, kalsiyum, magnezyum, manganez, demir ve sodyum, içerir. Garlic contains potassium, phosphorus, zinc, sulfur, selenium, calcium, magnesium, iron, sodium, and vitamins A and C (Ansary et al., 2020). Garlic contains polyphenols, flavonoids, polysaccharides, sulfur-containing compounds, and amino acids (Gorinstein et al., 2018; Setiawan et al., 2005). Due to its phenolic components, garlic has potent antioxidant properties (Petropoulos et al., 2018). Black garlic is produced by heat treatment of fresh garlic under controlled high-temperature conditions (60-90 °C) and high relative humidity (50-95%) without any extra treatment or additives (Zhang et al., 2016).

Garlic has many health benefits such as antioxidant, antifungal, hypoglycemic, antibacterial, anti-inflammatory, antiatherosclerotic and anticancer (Marchese et al., 2016; Sobenin et al., 2016; Mukthamba and Srinivasan, 2015). Sulfur compounds provide most of these beneficial effects of fresh and black garlic. Sulfur compounds are responsible for both the pungent odor of garlic and its medicinal effects (Jangam et al., 2014) and contain numerous bioactive components. Garlic constituents are divided into sulfur-containing compounds and non-sulfur-containing compounds (Anadón et al., 2016). More than half of the total organic sulfur compounds in a garlic clove are S-alkyl-L-cysteine sulfoxides (S-alk(en)yl-l-cysteine sulfoxides), as well as alliin ((+)-S-(2-propenyl)-L-cysteine sulfoxide), methinin ((+) - S-methyl-L-cysteine sulphoxide), isoalliin ((+) - S-trans- (1-propenyl) - L-cysteine sulphoxide), propiin ((+) S-propyl- L-cysteine sulphoxide) and trace amounts of ethyne ((+) - S-trans-ethyl- L-cysteine sulphoxide) (Krest et al., 2000; Kubec et al., 2009; Ramirez et al. 2017).

S-alkyl-L-cysteine sulfoxides are odourless compounds, but they produce many volatile products after garlic is crushed. When cell integrity is compromised or during storage, the enzyme alliinase is released from vacuoles, and hydrolysis of S-alkyl-L-cysteine sulfoxides (S-alk(en)yl-l-cysteine sulfoxides) occurs (Krest et al., 2000). The two sulfenic acid molecules formed then condense to form a thiosulfinate molecule (such as allicin formed from alliin), and these sulphides react with many other volatile compounds to form vinyldithiins and ajoenes (Krest et al., 2000; Ramirez et al., 2017). γ-glutamyl peptides are also thought to contribute to the

formation of new S-alkyl-L-cysteine sulphoxides compounds (Sendl, 1995). Studies have shown that both S-alkyl-L-cysteine sulfoxides and thiosulfinates positively affect health (Borlinghaus et al., 2014;). Therefore, the content of S-alkyl-L-cysteine sulphoxides is generally accepted as an indicator of garlic quality (Krest et al., 2000; Sendl, 1995). Various changes occur during the fermentation stages of black garlic production. The most important of these changes is the transformation of alliin into S-allyl cysteine (SAC) compound with antioxidant potential, not allicin, depending on the fermentation applied.

In this study, changes in the organosulfur content of white garlic and black garlic from Kahramanmaraş and Gaziantep were determined by liquid chromatography-electrospray ionization-tandem mass spectrometry (LC/ESI-MS/MS). This study aimed to determine the changes in organosulfur compounds in fresh and black garlic grown in Kahramanmaraş and Gaziantep provinces.

MATERIALS AND METHODS

Black garlic samples

In this study, Gaziantep and Kahramanmaraş fresh garlic, the most consumed garlic varieties in Turkey, and black garlic produced from these varieties at 65°C and 85% humidity in 24 days were used. A humidity chamber (Memmert HCP105) was used in black garlic production.

Analysis of the sulfur compounds

The extractions were performed using the method available by Sasmaz et al. (2022) with minor changes. The OS compounds were analyzed by using LC–DAD–ESI– MS/MS (Agilent 1260 HPLC; Agilent Technologies, Santa Clara, CA, USA) in a positive ionization mode utilized with the following parameters: drying gas of N2 at 12 L/min, capillary temperature of 400◦C, and nebulizer pressure of 45 psi of ESI/MS detection. The analyses were performed using a Phenomenex Luna reversed-phase C-18 column with a 4.6 mm×250 mm×5 m dimension. Two mobile phases were employed for the analysis: solvent A, consisting of a mixture of water and formic acid in a ratio of 99:1 (v/v), and solvent B, which was prepared by combining acetonitrile and solvent A in a ratio of 60:40 (v/v). Standard curves were computed based on the commercial standards at the concentrations existing in the extracts (1–100 mg/L) with R2 values higher than 0.99

Statistical data analysis

Data were assessed with the analysis of variance in the SPSS 20 package program at 95% confidence interval ($p < 0.05$) (SPSS Inc., Chicago, IL, USA). Duncan's multiple comparison test was utilized to decide the significance of the differences between the means.

RESULTS AND DISCUSSION

Sulfur compounds are sulfur-containing organic molecules associated with the pungent odors of allium vegetables, such as onions and garlic. The health benefits of garlic are due to the bioactive constituents, particularly sulfur compounds that cause bitterness. Garlics contain sulfur compounds such as alliin, allicin, and ajoene (Raghu et al.,2012). Sulfur compounds can be grouped into two categories water-soluble and oil-soluble compounds. Although water-soluble compounds constitute a small portion of garlic, they are thought to be the primary bioactive components for the prevention of cancer (Fukushima et al., 2001). Allium plants are recognized for producing a variety of cysteine sulfoxide complexes, including alliin, propiin, and methiin (Rose et al., 2005). When the tissues of Allium plants are broken down, the cysteine sulfoxides react with an enzyme called alliinase to form thiosulfinates such as allicin. Allicin's primary metabolic products are diallyl sulfides, diallyl di-sulfides, diallyl tri–tetra-sulfides, and sulfur dioxides (Fukushima et al., 2001). SAC is an essential bioactive compound with a significant pharmacological effect in black garlic (Bae et al., 2012). It is an odorless, stable, water-soluble compound with a high antioxidant capacity.

Differences in sulfur compounds of black garlic produced from fresh garlic were determined (Table 1 and Figure 1). The most important of these changes is the conversion of alliin into S-allyl cysteine (SAC) compound with antioxidant potential, not allicin, depending on the fermentation applied. SAC content was determined to be increased in black garlic compared to fresh garlic. It was determined that SAC content increased 4-fold in black garlic compared to fresh garlic. These findings are consistent with previous studies that have reported SAC content in black garlic to be five to six times higher than in fresh garlic. (Bae et al., 2012; Wang et al., 2012). Our study is consistent with the literature.

Table 10: Sulfur compounds of fresh and black garlic (g/100 g)

Sulfur compounds	Abbreviations	Rt (min)	Precursor ion (m/z)	Product ion (m/z)	Gaziantep Fresh Garlic	Kahramanmaraş Fresh Garlic	Gaziantep Black Garlic	Kahramanmaraş Black Garlic
γ-l-glutamyl-S-methyl-l-cysteine	GSMC	10,18	265,1	136.09, 119.09	2,61±0,10^{d}	3,02±0,06^{c}	7,24±0,88^{a}	5,40±0,34^{b}
(+)-S-allyl-l-cysteine	SAC	3,13	162,1	145.05, 73.10	3,61±0,06^{c}	3,84±0,22^{c}	10,43±0,35^{b}	15,32±0,54^{a}
(+)-S-allyl-l-cysteine sulfoxide	Alliin	6,93	178.1	88.1	2,55±0,04^{a}	1,26±0,12^{c}	1,75±0,02^{b}	1,11±0,81^{c}
Allicin	Allicin	55,3	163.2	73.2, 41.1	22,85±0,33^{a}	11,16±0,14^{b}	0,20±0,01^{d}	0,31±0,02^{c}
γ-l-glutamil-S-allyl-l-cysteine	GSAC	19,13	291.2	162,2, 144,8	0,86±0,02^{d}	1,74±0,12^{c}	2,63±0,08^{a}	2,44±0,01^{b}
γ-l-glutamil-phenylalanine	γGPA	24,55	295.3	178.0, 88.0	1,09±0,02^{a}	0,91±0,05^{b}	0,68±0,03^{c}	0,57±1,4^{d}
(3R,5S)-5-methyl-1,4-thiazane-3-carboxylic acid	Cycloalliin	4,89	178,23	88.08, 91.04	0,47±0,03^{c}	0,29±0,01^{d}	3,95±0,31^{b}	4,55±0,17^{a}
(+)-S-(trans-1-propenyl)-l-cysteine sulfoxide	Isoalliin	3,02	178,2	88.00, 160.10	0,96±0,09^{c}	0,75±0,01^{d}	4,56±0,05^{b}	5,68±0,44^{a}

$^{a-d}$Different letters in the rows represent statistically significant differences (P<0.05).

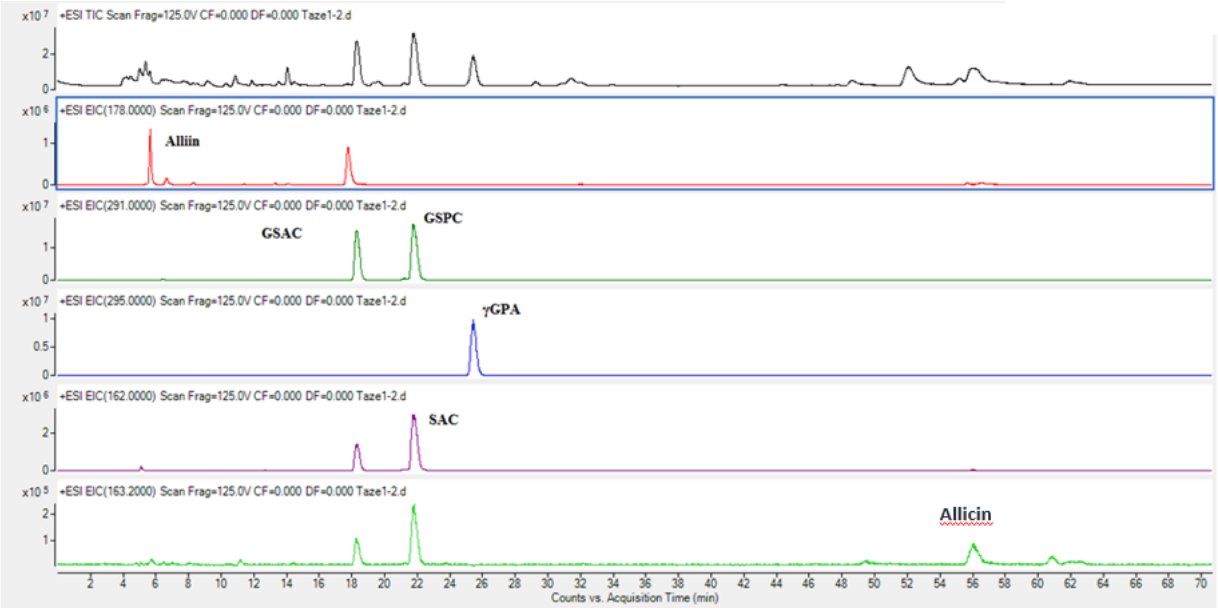

Figure 7: Chromatograms of organosulphur compounds

On the other hand, we observed a decrease in allicin content in black garlic compared to fresh garlic. Allicin is an important sulphur compound found in garlic known for its health benefits. It is produced when garlic cloves are mechanically damaged, leading to alliin breakdown by the enzyme alliinase. Fresh garlic has been found to have a higher allicin content compared to black garlic. In particular, the allicin content of fresh garlic grown in Gaziantep and Kahramanmaraş regions was 22.85 g/100 g and 11.16 g/100 g, respectively. In contrast, the allicin content in black garlic produced from garlic grown in these regions was significantly lower and was determined as 0.20 g/100 g and 0.31 g/100 g, respectively.

CONCLUSIONS

In conclusion, our study revealed that producing black garlic from fresh garlic leads to changes in sulphur compounds. The amounts of sulphur compounds in garlic grown in different regions were found to be different. While allicin content decreased in black garlic produced from fresh garlic grown in both regions during the production process, SAC content increased significantly in black garlic. In general, when the amount of sulphur compounds is examined, it is determined that garlic grown in the Gaziantep region has a higher amount of sulphur compounds in fresh garlic . While allicin is dominant in fresh garlic, it has been determined that SAC (S-allyl cysteine) compound is dominant in black garlic.These findings contribute to our understanding of the differences between fresh garlic and black garlic in terms of sulphur compound profiles and potential health benefits.

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Comparative evaluation of bioactive compounds changes from white to black garlic

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ABSTRACT

Garlic (*Allium sativum L.*) is a plant species in the Liliaceae family and has been used in the treatment of many diseases since ancient times due to its antioxidant, anticancer, antibacterial and antifungal effects. Garlic contains carbohydrates, proteins, amino acids, fibrous substances, fatty acids, phenolic compounds, vitamins, mineral substances and many sulfur compounds (allicin, alliin and ajoene). Black garlic is produced from fresh garlic by heat treatment without additives under controlled temperature and humidity. Numerous non-enzymatic browning reactions take place, including the Maillard reaction, oxidation of phenols, degradation of macromolecules and caramelization. Black garlic has a higher bioactive compound and nutrient content than fresh garlic. Black garlic has a higher protein content and a higher mineral content than fresh garlic. There are large changes in polyphenol content, total phenolic content increases during black garlic production, it contains high levels of polyphenols and its antioxidant capacity increases. There are very significant changes in the amount of organosulfur compounds, S-allyl cysteine (SAC) increases from 20-30 mg/g in fresh garlic to about 5-6 times in black garlic. Black garlic shows about 8-10 times higher in vitro and in vivo anti-inflammatory activity than fresh garlic. It shows protective effects on liver and cardiovascular diseases. Black garlic shows beneficial effects on memory and nervous system, anticancer antiallergic activity and antidiabetic effect.

Keywords: *Allium sativum L* , black garlic, phenolic

Antimicrobial activity of cell free supernatants of *lactiplantibacillus* plantarum strains at different growth conditions

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ABSTRACT

Among many *Lactobacillus* strains, *Lactiplantibacillus plantarum* (*L. plantarum*) is one of the species commonly used in many fermented foods as probiotic microbial starter. Many *L. plantarum* strains have a strong antimicrobial activity by producing different antimicrobial compounds such as organic acids, bacteriocins, phenylacetic acid, peptides, hydrogen peroxide, diacetyl, and fatty acids that are important for food biopreservation and human body with variable spectrum of action. So, the antagonistic activity of cell-free supernatants (CFS) of selected *L. plantarum* strains could contribute to food preservation as a natural antimicrobial agent. The objective of this study was to investigate the antimicrobial activity of CFS of *L. plantarum* which was grown at different concentrations of olive leaf extract (OLE) (1%-0.2%) and cell density (1%-3%). Lactic acid bacteria (LAB) counts varied between 8.21-9.6 log CFU/mL at different OLE concentrations and ranged from 8.40 to 10.02 log CFU/mL for different cell concentrations. The highest antimicrobial activity of CFS was found at a concentration of 1% cell density. The inhibition zone diameters of CFS were measured between 8.14-12.25 mm against *K. pneumoniae*, and *B. subtilis*. However, CFS did not show any antimicrobial activity against *E. coli* and *S. aureus*. In addition, antibiotic resistance profiles of CFS were analyzed using different types of antibiotics such as penicillin, ampicillin, sulfamethoxazole-trimethoprim, streptomycin, and ciprofloxacin. *K. pneumoniae* and *B. subtilis* strains showed only resistance to penicillin and ampicillin. However, these microorganisms became sensitive to these antibiotics when CFS of *L. plantarum* strains were added to the growth medium.

Keywords: Antimicrobial activity, cell-free supernatants, *L. plantarum*

Niosomes as nanocarriers for encapsulation of food ingredients

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ABSTRACT

Bioactive compounds, which are intensely found in plants and foods, have an important place in human and animal diets due to their antioxidant, anti-inflammatory, antibacterial and antiviral effects, prevention of many diseases and positive effects on health. In order to see the favorable effects of functional food components used for the enrichment of foods, it should be ensured that the body takes sufficient level. In addition, adding functional food ingredients directly to foods can adversely affect sensory properties such as color and taste. Carrier systems provide protection to components, which have antioxidant, antimicrobial and many other functional properties from chemical and biological degradation during processing, storage and direct usage in pure form. Nanocarriers and nanoencapsulation techniques have recently used for loading bioactive compounds in the food industry. Niosome is a vesicular nanocarrier that can be loaded with hydrophilic, lipophilic and amphiphilic components. It was developed initially as an alternative controlled drug delivery system to liposomes. It has a double layer structure consisting of non-ionic surfactant and lipid such as cholesterol. It has many advantages including protecting the functional ingredient from chemical or biological degradation during processing, homogeneous composition, lower production cost, variety of surfactants, long shelf life, easy storage, processing, and low toxicity. These properties of niosomes are important advantages for their applications in the food industry including encapsulation of bioactive substances such as phenolic, antioxidant and antimicrobial compounds. This review aims to give general information about the structure, formation, characterization of niosomes and their potential use in the food industry.

Effect of different *Lactobacillus* species on fermented beverage from chickpea and date

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ABSTRACT

There is a growing consumer demand for the consumption of plant-based alternative products that have many functional and health-promoting properties. Recently, there has been increased interest in plant-based milk and cow's milk substitutes, which are water-soluble extracts made from legumes, grains, or edible plant seeds. Chickpea is rich in a variety of nutrients including protein, mineral, vitamin, and fiber content. In this study, it was aimed to develop a chickpea and date based fermented beverage with *Lactobacillus acidophilus* (LA), *Lactobacillus casei* (LC), *Lactobacillus delbrueckii* (LD), and *Lactoplantibacillus plantarum* (LP) species. For this purpose, the influence of fermentation on the physicochemical properties (brix, pH, total acidity, colour), antioxidant capacity and total bacteria count (TBC) was examined at the end of 3 days of fermentation. The brix value was too close as 13.27±0.18 for all samples and 14.60±0.53 for the control sample (non-inoculated bacteria). pH value was measured as 4.27±0.01, 3.60±0.01, 3.74±0.01, 3.23±0.01, and 3.61±0.02 for the control, LP, LC, LA, and LD samples, respectively. Total acidity values for control, LC, LP, LA, and LD samples were calculated as 0.11±0.002, 0.18±0.002, 0.21±0.002, 0.39±0.002 and 0.22±0.001, respectively. While antioxidant activity is between 368.9 and 460.4 µmol Troloks/L with the DPPH method, it is between 790.9 and 893.6 µmol Troloks/L with the ABTS method. 3-Methylpentan-1-ol was found to be relatively higher among other volatile compounds identified in the samples. 2-pentyl furan, hexanoic acid, hexanol, and acetic acid were also determined as other major volatile compounds. TBC varied between 8.85-9.31 log CFU/mL in samples with added LD, LP, LC, and LD. In the control sample (non-inoculated), it was determined as 7.08 log CFU/mL. As a result of our study, fermentation has shown an important impact on the quality of the chickpea milk with LAB strains.

Keywords: chickpea milk, date, fermentation, lactic acid bacteria.

Application of anthocyanin extracts encapsulated by double emulsion method to ice cream

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ABSTRACT

The objective of this study was to produce ice cream using anthocyanin extracts encapsulated by double emulsion method. Firstly, the inner, oil and outer phases of the double emulsion were prepared with anthocyanin extracts or water (control samples), sunflower oil containing PGPR and 0.25% guar gum (w/v) and 6% inulin (w/v), respectively. The prepared emulsions were analyzed in terms of pH, color, encapsulation efficiency and stability, total anthocyanin, and phenolic content for 14 days. There was a decrease in L* values of emulsions during the storage time. Encapsulation efficiency of emulsions was found to be higher than 97.8%. Ice creams were produced using prepared double emulsions and pH, Brix, titration acidity, overrun, viscosity, texture, color, melting time, total anthocyanin and phenolic contents were determined for 60 days. Physicochemical properties of double emulsion-based ice creams were compared to control ice creams prepared as low fat, regular fat, and low fat with anthocyanins. The highest overrun (%) and viscosity (cP) values were 35.63±0.68 and 1977.13±2.92 in the double emulsion–based ice cream and in ice cream containing anthocyanin extract, respectively. The highest melting time (2930.33±55.29 s) was obtained in the control ice cream with anthocyanin extract. Results showed that double emulsions containing anthocyanin extracts can be used for ice cream production.

Keywords: Anthocyanin, double emulsion, encapsulation, ice cream.