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FOOD IN HEALTH AND DISEASE

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Sveučilište Josipa Jurja Strossmayera u Osijeku, Prehrambeno-tehnološki fakultet Osijek,
Franje Kuhača 18, 31000 Osijek, Hrvatska

Tehnička priprema i dizajn:
Damir Alihodžić (Tuzla, BiH), Ivana Lauš (Osijek, Hrvatska), Kenan Biberkić (Tuzla, BiH)

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SADRŽAJ / *CONTENT*

THE RELATIONSHIP BETWEEN FERRITIN LEVELS AND INDICATORS OF NUTRITIONAL STATUS IN CHILDREN AND ADOLESCENTS WITH ANOREXIA NERVOSA

Sara Galić[1*], Orjena Žaja[2-5], Ines Banjari[1,6]

[1]University of Mostar, Faculty of Agriculture and Food Technology, Biskupa Čule bb, 88000 Mostar, Bosnia and Herzegovina
[2]University Hospital Split, Pediatric Department, Spinčićeva ulica 1, 21000, Split, Croatia
[3]University Hospital Center, Pediatric Department & Center for Eating disorders, Klinički bolnički centar Sestre milosrdnice, Vinogradska cesta 29, 10000 Zagreb, Croatia
[4]University of Zagreb, School of Dental Medicine, Gundulićeva ulica 5, 10000 Zagreb, Croatia
[5]University of Split, School of Medicine, Šoltanska ul. 2A, 21000 Split, Croatia
[6]Josip Juraj Strossmayer University of Osijek, Faculty of Food Technology Osijek, Franje Kuhača 18, 31000 Osijek, Croatia

original scientific paper

Summary

Anorexia nervosa (*AN*) is a complex and potentially life-threatening eating disorder characterized by intense fear of weight gain and severe weight loss. This retrospective study included 58 patients treated at the Pediatric Department & Center for Eating Disorders, Sestre milosrdnice University Hospital Center. The objective was to assess the concentration of ferritin in relation to indicators of nutritional status and clinical characteristics in children and adolescents with *AN*. Only 2% of patients were diagnosed with the bulimic-purgative *AN* (*BP-AN*), while 5% had Eating Disorder Not Otherwise Specified (*EDNOS*), not otherwise categorized as atypical *AN* (*AAN*). Approximately 46% presented with a combination of restrictive-type *AN* and *EDNOS*. The average percentage of body weight loss was 20.46%, with the maximum recorded weight loss of 23 kg. In contrast, one patient lost no weight. The duration of illness prior to the diagnosis was longer in patients with classic *AN* compared to those with *AAN*, although the rate of weight loss was higher in the *AAN* group. This study also explored ferritin as a potential biomarker in *AN*, aiming to contribute to a better understanding of its pathophysiology and to identify novel diagnostic and therapeutic approaches.

Keywords: Anorexia nervosa, ferritin, weight loss, nutritional status

Introduction

In recent years, *AN* has increasingly taken hold in our society, particularly among young people. This eating disorder is characterized by severe body weight (*BW*) loss resulting from intentional and extreme restriction of food intake, without any known organic cause. Its prevalence continues to rise, representing a serious public health concern. Due to a distorted perception of their own body image, individuals affected by *AN* often conceal the problem and refuse to seek help, which makes the disorder especially complex and hinders both early detection and the treatment process itself (Žaja, 2014). Unfortunately, *AN* is frequently misunderstood as a lifestyle choice rather than a serious medical condition requiring professional intervention. The development of the disorder is generally associated with the family environment, media exposure, and engagement in rapid dieting practices.

Headlines such as “Battle with Fat,” “One-Day Super Diet,” “Lose 5 kg in Three Days,” and many others like them that scream from the covers of highly popular fashion magazines, along with the well-known phrase “You can never be too rich or too thin,” highlight today’s priorities, especially among women. *BW* regulation is increasingly common among young people, but unfortunately not as a result of health concerns, rather as a reaction to the omnipresent cult of thinness, which has been largely perpetuated through the media (Banjari et al., 2011).

Somatic and metabolic complications are extremely serious and, at times, life-threatening. Therefore, it is not surprising that there is a growing interest in identifying biomarkers that could facilitate the early detection of *AN*. Ferritin is increasingly mentioned in this context, as a significant number of studies point to the presence of hyperferritinemia in the restrictive type of *AN*. However, the reason behind such high ferritin levels in affected individuals remains unclear. Ferritin levels tend to return to the reference range as patients begin to gain *BW*, leading to the so-called spontaneous remission of ferritin levels in the body (Wanby et al., 2016; Teixeira et al., 2023). This study examined the potential role of ferritin as a biomarker of *AN*. The aim was to investigate the concentration of ferritin in relation to nutritional status and the clinical presentation of children and adolescents diagnosed with *AN*. The research was conducted in collaboration with the Pediatric Department & Center for Eating Disorders, Sestre milosrdnice University Hospital Center.

[*]Corresponding author: sara.galic@aptf.sum.ba

Participants and methods

Aim of the Study

The aim of this study was to examine the concentration of ferritin in relation to the nutritional status and clinical presentation of children and adolescents diagnosed with *AN*.

Participants

The study included 58 children and adolescents aged 9 to 18 years who were hospitalized at the Department of Gastroenterology, Pediatric Clinic, University Hospital Center "Sestre milosrdnice" in Zagreb with a diagnosis of *AN*. Study includes ethical approval from the ethics committee and official authorization for the use of medical records. The diagnosis of the eating disorder was made according to the current criteria for the given time period, specifically the *DSM-IV* criteria – *Diagnostic and Statistical Manual of Mental Disorders* (American Psychiatric Association, 2013). The research was designed as a retrospective observational study, without a control group.

Methods

Treatment data were collected from the medical records of children and adolescents available for all 58 patients treated at the Center over the past 10 years. Anthropometric measurements and the results of biochemical blood sample analyses were collected at the time of hospitalization at the Center. Among the blood parameters, iron status was assessed with a special focus on ferritin concentration in relation to *BW*, percentage of weight loss, application of nutritional support, and the presence of metabolic complications. All biochemical analyses were performed at the Clinical Institute of Chemistry of the Pediatric Department & Center for Eating Disorders, Sestre milosrdnice University Hospital Center as part of the clinical assessment of the patients.

The data were obtained through physical examination and anthropometric measurements of *BW*, measured using a mechanical beam scale (Seca, UK), and body height (*BH*), measured with an anthropometer while barefoot.

The year of disease onset and its duration in months were monitored, as well as *BW* before the illness. Weight loss from the onset of illness (% loss) was calculated using the following formula:

$$\% \text{ loss} = [(\text{BW before anorexia} - \text{BW}) / \text{BW before anorexia}] \times 100$$

Body Mass Index (*BMI*) was determined using the NHANES percentile curves for body weight by age (NCHS, 2002).

Statistical Analysis

Frequencies were reported for nominal variables. The Shapiro-Wilk test was used to analyze the normal distribution of variables. If the distribution of continuous variables did not deviate from normal, the arithmetic mean and standard deviation were used to present central tendency and dispersion, and parametric tests were used for comparisons. If the distribution of continuous variables was not normal, the median and range were used for presentation, and non-parametric tests were used for comparison Statistical analysis and graphical presentation of data were performed using the software system R Core Team (2023) and Microsoft Excel for Microsoft 365 MSO (Version 2111. Microsoft Corporation, Redmond, WA, USA).

Results and discussion

Out of all 58 patients included in the analysis, 56 were female and 2 were male. As a limitation of this study, we can state the gender imbalance considering that we have a higher percentage of female participants (96.6%). Since certain data for some patients were unavailable, missing values for numerical variables were replaced with the average value, and missing values for categorical variables were replaced with the most frequent category.

The sample under investigation predominantly consisted of female patients, which is consistent with other studies (Petranović et al., 2014) and with the higher prevalence of *AN* among adolescent girls.

In addition to sex, numerical values were recorded for the patients, including age (years), duration of illness since diagnosis (months), % loss, *BMI* (kg/m²), and ferritin levels in the blood (μg/L). For all variables, the basic descriptive indicators are provided using the following notations: *M* – mean value, *C* – median value, *SD* – standard deviation, *Min* – minimum value, *Max* – maximum value, *A* – skewness coefficient, *S* – kurtosis coefficient, *S-W* – Shapiro-Wilk test, and are presented in Table 1.

Table 1. Basic Descriptive Indicators of Numerical Variables in the Study

	M	C	SD	Min	Max	A	S	S-W	p
Age (years)	14.36	14.50	2.16	9.50	18.00	-0.25	2.56	0.97	0.10
Duration of illness prior to admission (months)	8.54	7.00	7.63	1.00	40.00	2.28	9.01	0.76	0.00
Body weight loss (%)	20.46	20.46	9.62	-0.24	65.67	1.69	10.36	0.85	0.00
BMI (kg/m^2)	16.28	16.05	2.57	12.30	24.70	0.79	3.66	0.95	0.02
Ferritin level (µg/L)	133.65	126.50	95.71	14.50	641.00	2.57	14.68	0.79	0.00

It is clearly evident from the previous table that most of the numerical variables deviated from a normal distribution, with the exception of patients' age. It is also important to highlight the substantial variability observed in the variables related to duration of illness prior to admission, percentage of loss (%), and ferritin levels.

Patient age ranged from 9.5 to 18 years. Most patients were between the ages of 13 and 16. These results are also consistent with the available literature, which indicates that *AN* is most commonly diagnosed at the age of 13, that is, during adolescence (Peterson et al., 2019).

Distribution of patients according to the percentage of loss (%) approximately 20% of *BW* was lost by the majority of patients, while two patients experienced a loss (%) exceeding 40%. The greatest loss (%) was observed in a 15-year-old female patient, where the percentage reached 65.67%, and the illness was diagnosed slightly over a year after its onset.

The majority of patients had elevated serum ferritin levels. No patient showed ferritin levels below the minimum reference value. The highest ferritin level was recorded in a 15-year-old female patient, who also had the highest recorded loss (%).

In addition to the above values, other biochemical parameters were recorded in patients, such as: C-reactive protein (*CRP*), Leukocytes (*L*), Aspartate aminotransferase (*AST*), Alanine aminotransferase (*ALT*), Lactate dehydrogenase (*LDH*), Hemoglobin (*Hgb*), Iron (*Fe*), Unsaturated Iron Binding Capacity (*UIBC*), and Total Iron Binding Capacity (*TIBC*). These values were categorized into three groups: 0 for results within the reference values, 1 for results below the minimum reference value, and 2 for results above the reference values. Their distribution is shown in Table 2.

Table 2. Frequency of blood parameter levels in patients

Parametar	1 – below reference values n (%)	0 – within reference values n (%)	2 – above reference values n (%)	$\chi2$	df	p
CRP	1 (1.72%)	57 (98.28%)	-	54.07	1	< 0.001
L	-	43 (74.14%)	15 (25.86%)	13.52	1	< 0.001
AST	6 (10.34%)	51 (87.93%)	1 (1.72%)	78.45	2	< 0.001
ALT	7 (12.07%)	50 (80.21%)	1 (1.72%)	73.9	2	< 0.001
LDH	-	58 (100.00%)	-	-	-	-
Hgb	1 (1.72%)	56 (96.55%)	1 (1.72%)	104.31	2	< 0.001
Fe	-	55 (94.83%)	3 (5.17%)	46.62	1	< 0.001
UIBC	-	34 (58.62%)	24 (41.38%)	1.72	1	0.19
TIBC	-	27 (46.55%)	31 (53.45%)	0.28	1	0.60

Across all observed parameters, the majority of patients had values within the reference range. Only for the parameters *UIBC* and *TIBC* the number of patients with elevated values was equal to those with values within the reference range. The data clearly indicate that elevated ferritin levels cannot be explained by inflammatory processes in the body (*CRP* levels), impaired liver function (*AST* and *ALT*), or iron metabolism (*Hgb*, *Fe*, *UIBC*, *TIBC*), and were therefore not influenced by other factors.

The results of this study are consistent with those reported by Tran et al. (2013), who also observed elevated ferritin levels in the absence of inflammation or liver dysfunction. The authors noted that this is a frequent yet still insufficiently explained biochemical phenomenon. Similar conclusions were drawn in a more recent research by Teixeira et al. (2023), which further confirmed that high ferritin concentrations may be present in patients with AN without being associated with inflammatory markers or liver function disorders.

Although these findings indicate a statistically significant association between ferritin levels and the percentage of *BW* loss, the absence of a control group limits the possibility of drawing causal inferences. Thus, while ferritin may reflect aspects of nutritional status in patients with *AN*, it should not be interpreted as a causal biomarker. Further prospective studies with appropriate controls are needed to establish whether ferritin can serve as a valid and reliable diagnostic tool in this context. Until such validation is achieved, its use in clinical diagnostics of *AN* should be approached with caution.

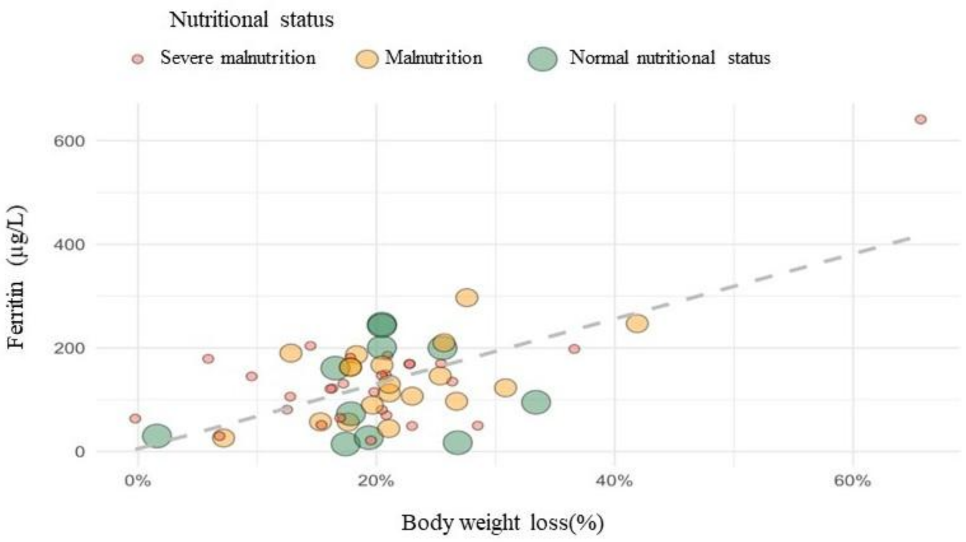

Figure 1. Graphical representation of the relationship between two variables of interest

Additionally, Figure 1 emphasizes the level of nutritional status, *BW* % loss and ferritin levels in patients with *AN*. What is particularly noteworthy is that nutritional status appears to correlate less strongly with ferritin levels than the percentage of loss (%). This is clearly evident in the distribution of each nutritional status group on the left and right sides of the graph. A similar result was reported in the study by Wanby et al. (2016), where no correlation was found between *BMI* and plasma ferritin concentration—neither in the entire patient group, nor in the subgroup of patients with *AN*, nor in the group with eating disorders without *AN*.

To examine the previously proposed hypothesis regarding the correlation between ferritin levels and the percentage of loss (%), a correlation test was conducted. Spearman's rank correlation coefficient between ferritin and loss (%) was 0.30 and it was statistically significant at a significance level of less than 5%. The correlation between ferritin and percentage of loss (%) was positive. In other words, patients with higher blood ferritin levels also had a higher percentage of loss (%).

Based on *BMI*, patients were categorized according to degree of malnutrition. Patients with a *BMI* below 16 kg/m² were classified as severely malnourished, those with a *BMI* between 16 and 18.4 kg/m² were categorized as malnourished, and those with a *BMI* between 18.4 and 24.9 kg/m² were considered to have a normal nutritional status. Figure 2 shows ferritin values within each of the three mentioned groups.

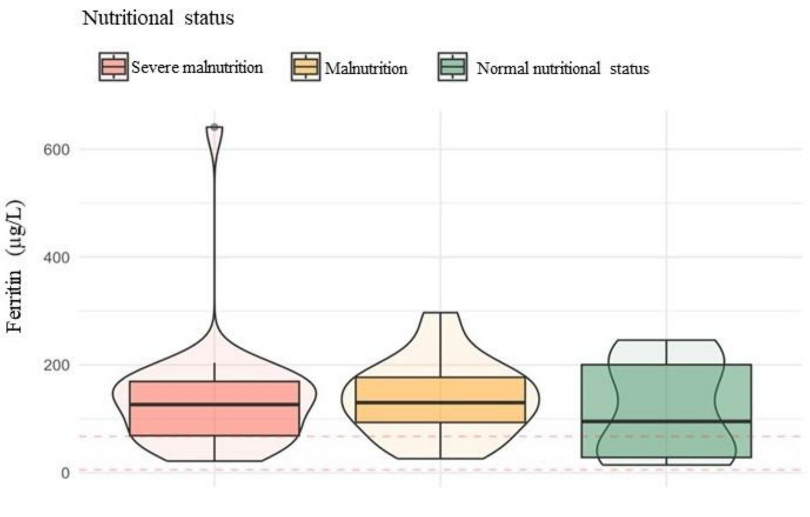

Figure 2. Blood Ferritin Levels According to the Degree of Malnutrition

Among the group of severely malnourished patients, a total of 7 out of 28 (25.00%) patients had ferritin levels within the reference range. A similar percentage was observed in the malnourished group, where 4 out of 19 (21.05%) patients had ferritin levels within the reference range. In the final group, those with normal nutritional status, 4 out of 11 (36.36%) patients had ferritin levels within the reference range.

Given that the ferritin distribution in the severely malnourished patient group markedly deviated from normality, differences were assessed non-parametrically using the Kruskal–Wallis test (Table 3).

Table 3. Results of the Kruskal–Wallis test for differences in ferritin levels among patients according to the degree of malnutrition

	C	(Min - Max)	χ^2	df	p
			0.49	2	0.78
Severe malnutrition	126.5	(21.6 - 641)			
Malnutrition	130	(26.1 - 297)			
Normal nutritional status	95.1	(14.5 - 246)			

Although ferritin levels were higher in malnourished and severely malnourished patients compared to those with normal nutritional status, no statistically significant differences in ferritin levels were observed among the groups based on the degree of malnutrition.

Conclusion

This study showed that the majority of patients with *AN* have elevated ferritin levels in their blood. It is particularly noteworthy that no patient had ferritin levels below the minimum reference value. A 15-year-old female patient, who had the highest recorded ferritin levels, also experienced the greatest weight loss. Therefore, restoring ferritin to reference intervals is an important goal in the treatment of *AN* in children and adolescents, not only because it is an indicator of adequate *BW* but also because it is one of the main indicators that the body is recovering. This research makes a significant contribution to the growing body of evidence supporting ferritin as a reliable indicator of nutritional status in children and adolescents with *AN*. The results of this study confirm the hypothesis that ferritin levels correlate with nutritional status in this population, highlighting its importance in clinical assessment and monitoring of patients. Although further research is needed to fully understand the complexity of the relationship between ferritin and nutritional status in *AN*, these results represent a step forward in that direction. Overall, focusing on potential biomarkers in the future, such as ferritin, could have a significant clinical implications for early detection and monitoring of *AN*.

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MEDITERANEAN DIET PRACTICES AND CARDIOVASCULAR RISK AMONG STUDENTS OF HEALTH STUDIES FROM MOSTAR, SARAJEVO AND SPLIT

Amina Šeta[1*], Senka Dinarević[2], Arzija Pašalić[3], Nihad Fejzić[4], Irena Drmić Hofman[5], Lutvo Sporišević[6], Ljerka Ostojić[2], Ivan Ćavar[7], Antonela Matana[5], Mario Merendić[5], Mirela Lisičić-Konaković[6], Anes Jogunčić[8], Aida Pilav[8]

[1]University SSST, Unimed Polyclinic, Hrasnička cesta 3a, 71210, Ilidža, Sarajevo, Bosnia and Herzegovina
[2]Academy of Arts and Sciences of Bosnia and Herzegovina, Bistrik 7, 71000 Sarajevo, Bosnia and Herzegovina
[3]University of Sarajevo, Faculty of Health Studies, Stjepana Tomića 1, 71000 Sarajevo, Bosnia and Herzegovina
[4]University of Sarajevo, Veterinary Faculty, Zmaja od Bosne 90, 71000 Sarajevo, Bosnia and Herzegovina
[5]University of Split, University Department of Health Studies, Ruđera Boškovića 35, 21000 Split, Croatia
[6]Health Center of Sarajevo Canton, Vrazova 11, 71000 Sarajevo, Bosnia and Herzegovina
[7]University of Mostar, Faculty of Medicine, Kralja Petra Krešimira IV. bb Bijeli Brijeg, 88000 Mostar, Bosnia and Herzegovina
[8]Institute for Public Health of the Federation of Bosnia and Herzegovina, Tahtali sokak 17, 71000 Sarajevo, Bosnia and Herzegovina

original scientific paper

Summary

Introduction: The Mediterranean diet has been demonstrated to confer significant benefits in the prevention of cardiovascular disease. This study represents the first collaborative effort between researchers from Bosnia and Herzegovina and Croatia to examine the effects of the Mediterranean and non-Mediterranean dietary patterns on cardiovascular risk factors within the student population. The primary objective was to identify specific dietary behaviors and patterns that may contribute to a cardiovascular risk reduction. Materials and Methods: This cross-sectional study was conducted in 2024 and included 473 students from health studies faculties in three cities: Sarajevo, Mostar, and Split. Independent variables in the study were diet type (Mediterranean vs. non-Mediterranean), physical activity, and smoking. Dependent variables included lipid profile (cholesterol, triglycerides), blood pressure, and blood glucose levels. Results: Students from Split exhibited the highest consumption frequency of olive oil, fish, vegetables, and legumes consumption, which was statistically significant compared to students from Mostar and Sarajevo ($\chi^2 = 11.488$, $p = 0.022$). A statistically significant difference was also found in the consumption of animal fats (butter, cream, margarine) across the cities ($\chi^2 = 19.860$, $p = 0.003$). The average adherence score to the Mediterranean diet was highest among students from Split (3.80 points) and lowest among those from Sarajevo (2.77 points). Significant differences in cholesterol and triglyceride levels were observed only among female students across the cities (Kruskal-Wallis test, $p < 0.05$). Conclusion: A significant difference in cardiovascular risk factors was observed between students following the Mediterranean diet and those adhering to a non-Mediterranean diet. The Mediterranean diet demonstrated a beneficial effect on cardiovascular risk, as evident in the lipid profile. In contrast, non-Mediterranean diet, characterized by higher intakes of vegetable and animal fats, was associated with increased cardiovascular risk.

Keywords: Mediterranean diet, student population, lipid profile, Croatia, Bosnia and Herzegovina

Introduction

In contemporary research, diet and its influence on human health have become central topics in numerous global studies. A recent review highlights that dietary habits play a pivotal role in the prevention and management of cardiovascular diseases, which remain the leading cause of death worldwide (Lu et al., 2024). The Mediterranean diet, characterized by a high intake of fruits, vegetables, fish, whole grains, and olive oil, has been consistently linked to a reduced risk of cardiovascular disease, as evidenced by several studies (Widmer et al., 2014; Pant et al., 2024). This diet emphasizes the reduction of added sugars found in baked goods, ice cream, some granola bars, and sugary beverages like fruit juices and sodas, as well as alcohol, high-sodium foods, saturated fats, refined carbohydrates (such as white bread and rice), processed cheeses, and fatty or processed meats. The Mediterranean diet has been shown to be beneficial in preventing cardiovascular diseases (Critselis et al., 2021). Research on the Mediterranean diet has consistently demonstrated its association with favorable health outcomes, including a reduced incidence of heart disease, hypertension, type 2 diabetes, and improved lipid profiles (Scholl et al., 2012; Rosato et al., 2017).
In contrast, the non-Mediterranean diet, which is a traditional omnivorous diet rich in dairy products, meat, vegetables, and fruits, as well as characterized

*Corresponding author: aminagodinjak@gmail.com

by specific methods of food preparation and consumption, remains insufficiently explored in terms of its impact on cardiovascular health. While studies examining non-Mediterranean diets are limited, preliminary data suggest that they may offer potential health benefits, particularly when based on plant-derived sources and traditional preparation methods that minimize the use of processed foods (Hotz & Gibson, 2007). Given the relatively limited research in this area, there is a clear need for further investigation, underscoring the importance of conducting this study.
Given the increasing global concern regarding cardiovascular diseases, particularly among younger populations, and the significant role of diet in their prevention and management, this study aims to contribute to the existing body of scientific knowledge by investigating the specific effects of Mediterranean and non-Mediterranean diets on cardiovascular risk factors among health studies students. Health science students were chosen for this evaluation due to their generally healthier dietary patterns and lifestyles compared to students in other academic fields (de-Mateo-Silleras et al., 2019). This study represents the first collaborative effort between researchers from Bosnia and Herzegovina and Croatia to explore the impact of Mediterranean and non-Mediterranean diets on cardiovascular risk among students in three cities: Split, a Mediterranean city on the Adriatic coast; Sarajevo, a non-Mediterranean city; and Mostar, situated between the two. Central and Eastern European countries, including those in the Balkans, exhibit the highest cardiovascular mortality rates in Europe (Townsend et al., 2016). In 2021, diseases of the circulatory system caused 1.71 million deaths in the European Union, accounting for 32.4% of all deaths. The cardiovascular disease death rate in Croatia was 36.8%, which is higher than the European average (Eurostat, 2017).
The findings of this research could have important implications for the development of dietary guidelines and the promotion of healthier lifestyles. The primary objective of this study is to identify specific dietary behaviors and patterns that contribute to the reduction of cardiovascular disease risk, as assessed through indicators such as blood pressure, lipid and glucose levels, and anthropometric measurements (e.g., body mass index and waist-to-hip ratio).

Materials and methods

This cross-sectional study was approved by the Ethics Committee of The Academy of Arts and Sciences of Bosnia and Herzegovina. A total of 473 students from health studies faculties in three cities participated in the research: Sarajevo (230 students; the University of Sarajevo-Faculty of Health Studies), Mostar (125 students: the University of Mostar-Faculty of Medicine), and Split (118 students; the University of Split-Faculty of Health Studies). The research was conducted between April and September 2025. Independent variables were: type of diet (Mediterranean vs. non-Mediterranean), physical activity, and smoking. Physical activity and smoking habits were determined by self-assesment. Dependent variables were: lipid profile (cholesterol, triglycerides), blood pressure, and blood glucose levels.
Type of Diet: Frequency of consumption of key components of the Mediterranean diet (e.g., fruits, vegetables, fish, olive oil) and the non-Mediterranean continental diet (e.g., red meat, dairy products, bread). The MEDAS Mediterranean Diet Adherence Screener (Annex 1) was used to assess dietary patterns. It is a valid and reliable tool for evaluating adherence to the Mediterranean diet across diverse populations (Schröder et al., 2011; Marendić et al., 2021; Bekar & Göktaş, 2023).

Biochemical analyses

Blood samples were collected in the early morning hours between 7:00 and 9:00 a.m. to reduce the influence of diurnal fluctuations on the physiological parameters. Participants instructed not to consume food, drink (except water) or smoke for at least 8 hours prior to testing. Capillary blood was used to measure fasting lipid status, i.e. total cholesterol, triglyceride and fasting glucose. Lipid status was measured with the Accutrend Plus device from Roche Diagnostics, Switzerland, according to the manufacturer’s manual. Blood glucose was measured with the Accu-Chek device from Roche Diabetes Care GmbH, Germany, according to the manufacturer’s instructions.

Body weight and height

Body weight was measured in the morning, before meals, and after emptying the bladder. A medical decimal scale with an accuracy of ±100 g was used to measure weight while the subject stands, without shoes and in light clothes, or in underwear.
Height is measured with an anthropometer consisting of a metal rod, so that the subject stands in an upright position on a flat surface, evenly distributed on both legs. The shoulders are relaxed, the heels are pulled up and the head is in the "frankfurt horizontal position".

Anthropometric measurement of waist and hip circumference

The waist and hip circumference is measured with a non-elastic measuring tape. The waist circumference is measured midway between the lower ribcage and the front upper ribcage, with the subject standing evenly on both legs while breathing normally. It is recommended that the examiner sits opposite the subject so that the measuring point is at eye level. The hip circumference is measured at the level of the widest part of the gluteal muscle.

The waist-to-height ratio (WHtR)

The waist-to-height ratio (WHtR) is calculated as the ratio of body circumference in cm divided by body height in cm. The default value for WHtR is considered 'no increased risk' (WHtR <0.5); 'increased risk' (WHtR ≥0.5 to <0.6) and 'very high risk' (WHtR ≥0.6). The threshold value for WHtR is set at 0.5. Similarly, WC ≥90 cm for men and ≥80 cm for women were considered as 'cut-off values'.

Blood pressure

A mercury sphygmomanometer manufactured by Bokang (Wenzhou Bokang Instruments Co., Ltd., China) was used to measure blood pressure. The systolic and diastolic blood pressure values were measured with the device with the appropriate cuff size on the upper arm in a sitting position and after 5 minutes of rest. The blood pressure of each participant was measured twice at intervals of 1–2 minutes and the average of the values was calculated.

Statistical Analysis

Statistical methods were employed to examine the relationship between independent variables (diet type, physical activity, smoking) and dependent variables (lipid profile, blood pressure, blood glucose). Multivariable regression analysis and ANOVA were utilized to assess the impact of the Mediterranean and non-Mediterranean diets on cardiovascular risk factors. The level of significance was < 0.5. Additionally, adherence to the Mediterranean diet was evaluated using the MEDAS score.

Results

The study included 473 health science students from three cities: Mostar (26.4%), Sarajevo (48.6%), and Split (24.9%). The gender distribution was predominantly female, comprising 87.3% of the participants, while 12.5% were male. Most students were in the 1st (30%), 2nd (31.5%), and 3rd (32.6%) years of study. The dietary habits and patterns of the studied student population are summarized in Table 1.

Table 1. General information about study participants

	Total	Sarajevo	Mostar	Split	p
Age	21.66 ± 4.3	21.2 ± 1.3	24.1 ± 7.2	19.9 ± 1.2	F=37.58 p<0.001
Sex *n, %* Male *n, %* Female *n, %*	473 (100) 59 (12.5) 414 (87.5)	33 (14.3) 197 (85.7)	10 (8.0) 115 (92.0)	16 (13.6) 102 (86.4)	x^2=3.16 p=0.206
Year at University I year *n, %* II year *n, %* III year *n, %* IV year *n, %* V year *n, %*	142 (30.0) 149 (31.5) 154 (32.6) 14 (3.0) 14 (3.0)	43 (18.7) 64 (27.8) 108 (47.0) 13 (5.7) 2 (0.9)	44 (35.2) 57 (45.6) 11 (8.8) 1 (0.8) 12 (9.6)	55 (46.6) 28 (23.7) 35 (29.7) 0 (0) 0 (0)	x^2=106.12 p<0.001
Smoking Yes *n, %* No *n, %*	149 (31.5) 324 (68.5)	62 (27.0) 168 (73.0)	48 (38.4) 77 (61.6)	39 (33.1) 79 (66.9)	x^2=5.090 p=0.078
Characteristics of smoking: Non-Smoker *n, %* Previous smoker *n, %* Social smoker *n, %* Smoker *n, %*	304 (64.3) 20 (4.2) 63 (13.3) 86 (18.2)	158 (68.7) 10 (4.3) 29 (12.6) 33 (14.3)	71 (56.8) 6 (4.8) 14 (11.2) 34 (27.2)	75 (63.6) 4 (3.4) 20 (16.9) 19 (16.1)	x^2=11.50 p=0.074
Physical activity* Never *n, %* Few times per month *n, %* Few times per week *n, %* Every day *n, %*	37 (7.8) 114 (24.1) 214 (45.2) 108 (22.8)	10 (4.3) 55 (23.9) 101 (43.9) 64 (27.8)	18 (14.4) 32 (25.6) 55 (44.0) 20 (16.0)	9 (7.6) 27 (22.9) 58 (49.2) 24 (20.3)	x^2=16.58 p=0.011

*at least one hour

Regarding the distribution across years of study, the majority of participants were in their second (31.5%) and third year (32.6%), followed by the first year (30.0%), with smaller proportions in the fourth (3.0%) and fifth year (3.0%). Among males, half were in their first year (50.8%), while fewer were in the second (20.3%), third (23.7%), and fourth year (5.1%), with no males in the fifth year. Female students were most frequently in the third year (33.8%) and second year (33.1%), followed by the first year (27.1%), with fewer in the fourth (2.7%) and fifth year (3.4%). The distribution across years differed significantly between males and females (χ^2 = 17.04, p = 0.002), indicating a statistically significant variation in year of study by sex.

Overall, 149 students (31.5%) reported that they smoke, while 324 (68.5%) were non-smokers. Among males, a significantly higher proportion were smokers (55.9%) compared to females (29.7%), (χ^2 = 4.923, p = 0.026).

When further examining smoking characteristics, 64.3% of the total sample were non-smokers, 4.2% were previous smokers, 13.3% were social smokers, and 18.2% were active smokers. Among males, 49.2% were non-smokers, while a larger portion reported active smoking (33.9%), and smaller proportions reported being social smokers (10.2%) or former smokers (6.8%). In contrast, 66.4% of females were non-smokers, 15.9% were active smokers, 13.8% were social smokers, and 3.9% were former smokers. The differences in smoking characteristics between male and female students were statistically significant (χ^2 = 9.576, p = 0.023).

Daily physical activity that lasted at least one hour, was most freaquent among students from Sarajevo, where almost third of them reported daily activities (p=0.011).

Regarding olive oil consumption, the student population from Split had the highest consumption frequency of olive oil (55.9%), which was statistically significant when compared to students from Mostar and Sarajevo (χ^2 = 7.92, p = 0.019). For vegetable consumption, Split also had the highest percentage of students consuming ≥2 servings of vegetables per day (33.9%), which was statistically significant in comparison to the students in Mostar and Sarajevo (χ^2 = 13.519, p = 0.009).

A statistically significant difference was found the for consumption of animal fats (butter, cream, margarine), which was the highest among students from Split (χ^2 = 19.860, p = 0.003). Regarding fish consumption (≥3 portions per week), Split had the highest consumption (21.2%), as compared to students from Sarajevo (13.2%), a statistically significant difference (χ^2 = 10.374, p = 0.035). In terms of legume consumption, students from Sarajevo had the lowest frequency of legume consumption per week, which was statistically significant as compared to students from the other cities (χ^2 = 16.462, p = 0.036).

The consumption of sweetened beverages was statistically highest among students from Sarajevo, while those from Split had the lowest consumption (χ^2 = 42.888, p = 0.0006). There was also a statistically significant difference in the consumption of tomato sauce between students from Split and Mostar, as well as between Split and Sarajevo (χ^2 = 11.488, p = 0.022). However, no statistically significant differences was found in the consumption of fruits and nuts between students from all cities.

Table 2. Comparsion of dietary habits and patterns of students from Sarajevo, Split and Mostar

City	Respondents	% of total respondents
Consumption of olive oil as the main source of fat in the preparation of daily meals		
Mostar		
Yes	52	41,6
No	73	58,4
Total	*125*	*100*
Sarajevo		
Yes	69	30
No	161	70
Total	*230*	*100*
Split		
Yes	66	55,9
No	52	44,1
Total	*118*	*100*
χ2 =22.429 (df=2; p=0.000) p<0.05		
Vegetable consumption in daily diet (1 portion cca. 200 gr)		
Mostar		
>=2 portions	24	19,2
0 portion	10	8,0
1 portion	91	72,8
Total	*125*	*100,0*
Sarajevo		
>=2 portions	40	17,4
0 portion	15	6,5
1 portion	175	76,1
Total	*230*	*100,0*

Table 2. Continued...

Split		
>=2 portions	40	33,9
0 portion	7	5,9
1 portion	71	60,2
Total	*118*	*100,0*
χ2 =13.519 (df=4; p=0.009) p<0.05		
Consumption of animal fats (butter, margarine or cream) (1 portion cca. 12 gr)		
Mostar		
0 portion	33	26,4
1 portion	75	60,0
2 portions	15	23,0
3 portions	2	1,6
Total	125	100,0
Sarajevo		
0 portion	48	20,9
1 portion	157	68,3
2 portions	22	9,6
3 portions	3	1,3
Total	230	100,0
Split		
0 portion	49	41,5
1 portion	62	52,5
2 portions	5	4,2
3 portions	2	7,0
Total	118	100,0
χ2 =19.860 (df=6; p=0.003) p<0.05		
Weekly fish or seafood consumption (1 serving approx. 150 gr)		
Mostar		
0 portion	32	27,6
1 portion	67	55,8
2 portios	21	17,5
Total	*120*	*100,0*
Sarajevo		
0 portion	73	33,2
1 portion	118	53,6
2 portios	29	13,2
Total	*220*	*100,0*
Split		
0 portion	20	17,7
1 portion	69	61,1
2 portios	24	21,2
Total	*113*	*100,0*
χ2 =10.374 (df=4; p=0.035) p<0.05		
Consumption of legumes		
Mostar		
>=3 portions	19	15,3
0 portion	13	10,5
1 portion	49	39,5
2 portions	43	34,7
Total	*124*	*100,0*
Sarajevo		
>=3 portions	21	9,1
0 portion	43	18,7
1 portion	106	46,1/
2 portions	60	26,1
Total	*230*	*100,0*
Split		
>=3 portions	18	15,3
0 portion	14	11,9
1 portion	43	36,4
2 portions	43	36,4
Total	*118*	*100,0*
χ2 =16.462 (df=7; p=0.036) p<0.05		
Daily consumption of sweetened beverages (1 cup = 100ml)		
Mostar		
>=3 portions	12	9,6
0 portion	55	44,0
1 portion	30	24,0
2 portions	28	22,4
Total	*125*	*100,0*

Table 2. Continued...

Sarajevo		
>=3 portions	13	5,7
0 portion	54	23,5
1 portion	126	54,8
2 portions	37	16,1
Total	*230*	*100,0*
Split		
>=3 portions	6	5,1
0 portion	53	44,9
1 portion	49	41,5
2 portions	10	8,5
Total	*118*	*100,0*
χ2 =42.888 (df=6; p=0.000) p<0.05		
Weekly consumption of tomato sauce and olive oil		
Mostar		
>2 portions	84	67,2
0 portion	3	2,4
1 portion	38	30,4
Total	*125*	*100,0*
Sarajevo		
>2 portions	153	66,5
0 portion	6	2,6
1 portion	71	30.9
Total	*230*	*100,0*
Split		
>2 portions	98	83,1
0 portion	2	1,7
1 portion	18	15,3
Total	*118*	*100,0*
χ2 =11.488 (df=4; p=0.022) p<0.05		

The average body mass index (BMI) across both sexes and all cities was 23.37 ± 7.96 kg/m², which falls within the normal weight range. No statistically significant differences was found for body weight, height, hip circumference, waist circumference, waist-to-hip ratio (WHR), or blood pressure values between students from these cities (Table 2). Additionally, no significant difference in systolic and diastolic blood pressure were found across the cities (ANOVA test). Among men, no significant differences in glucose, total cholesterol, or triglyceride levels were observed between the cities (Kruskal-Wallis H test). However, in women, significant differences in total cholesterol and triglyceride levels were found across the cities (Kruskal-Wallis test, $p < 0.05$).

Table 3. Anthropometric, blood pressure, total cholesterol, triglyceride and blood glucose measurements of students from Mostar, Sarajevo and Split

		Split	Mostar	Sarajevo	F	p
Body weight (kg)	Male	82.9 ± 11.9	73.3 ±13.9	82.4 ± 12.8	2.169	0.124
	Female	63.9 ± 9.8	65.5 ± 10.7	65.1 ± 10.8	0.595	0.552
Body height (cm)	Male	184.1 ± 7.9	180.7 ± 12.46	183.1 ± 6.3	0.547	0.582
	Female	170.2 ± 5.42	168.9 ± 14.6	167.9 ± 6.6	2.042	0.131
BMI (kg/m2)	Male	24.5 ± 2.70	22.3 ± 2.99	24.5 ± 4.05	1.606	0.210
	Female	22.6 ± 3.52	23.3 ± 3.95	23 ± 3.64	1.382	0.252
Waist circumference	Male	87.5±9	80.8±12.7	86.5±12	0.983	0.381
	Female	73.1±9.4	74.7±9.4	72.4±8.7	2.988	0.052
Hip circumference	Male	106.8±6.4	98.4±9.2	102.9±9.9	2.029	0.141
	Female	96.6±9.2	100.6±18	96.5±10.2	4.63	0.010
WHR	Male	0.5±0.1	0.5±0.1	0.5±0.1	0.326	0.723
	Female	0.4±0.1	1.7±11.4	0.4±0.1	1.597	0.204
Systolic BP	Male	124.1±15.5	120.5±10.7	124.2±13.6	0.307	0.737
	Female	115.1±12.4	115.8±9.1	117.5±12	2.713	0.068
Dyastolic BP	Male	72.1±10.2	74.5±6.9	77.8±7.5	2.706	0.076
	Female	74.7±8.7	72±14.2	72.7±13.1	1.324	0.267
Glucose	Male	5.5±0.4	5.9±0.9	5.6±0.8	1.109	0.574
	Female	5.5±0.6	5.7±1.1	5.3±1.1	24.83	<0.001
Cholesterol	Male	3.9±0.6	4.5±0.9	4.6±1.2	3.235	0.198
	Female	4.2±0.5	4.1±0.6	4.6±1.1	27.871	<0.001
Triglycerides	Male	1.2±0.6	1.4±0.6	2.6±1.7	19.322	<0.001
	Female	1.4±1.2	1.2±1	2.4±1.8	75.953	<0.001

The student population from Split demonstrated the highest level of adherence to the Mediterranean diet, based on the MEDAS score, particularly in terms of olive oil use, vegetable intake, and fish consumption (Table 3). The ANOVA test was applied to the MEDAS scores, yielding an F-statistic of 0.0 and a p-value of 1.0, indicating no significant differences between the cities.

The odds ratio for fish consumption (Split vs. Sarajevo) was calculated as OR = 1.77, suggesting that students from Split have a 77% higher likelihood of consuming fish ≥3 times per week as compared to their counterparts from Sarajevo. The average MEDAS score was highest in Split (3.80 points) and lowest in Sarajevo (2.77 points). However, no statistically significant difference was found in the MEDAS scores between the cities.

Table 4. Adherence to the Mediterranean diet based on the MEDAS score between students from Mostar, Sarajevo and Split

	Mostar		**Sarajevo**		**Split**	
MEDAS score	Mean	SD	Mean	SD	Mean	SD
	3.37	1.93	2.77	1.78	3.80	1.83
	Median	*IQ range*	*Median*	*IQ range*	*Median*	*IQ range*
MEDAS score	3.0	(2.0-5.0)	2.0	(1.0-4.0)	4.0	(2.0-5.0)

Table 5. Categorisation of students from Mostar, Sarajevo and Split based on the adherence to the Mediterranean diet

City	**Low adherence**		**Moderate adherence**		**High adherence**	
Mostar	107	85,6%	17	13,6%	1	0,8%
Sarajevo	209	90,9%	21	9,1%	0	0,0%
Split	99	83,9%	17	14.4%	2	1,7%

Table 6. Categorization of nourishment status of students from Mostar, Sarajevo and Split based on their calculated Body Mass Index

	Mostar	**Split**	**Sarajevo**	**P**
Underweight	8 (6.5%)	5 (4.3%)	6 (2.6%)	x^2=12.019 p=0.062
Normal weight	88 (71.5%)	88 (75.9%)	164 (71.6%)	
Overweight	27 (22.0%)	19 (16.4%)	45 (19.7%)	
Obesity	0 (0%)	4 (3.4%)	14 (6.1%)	

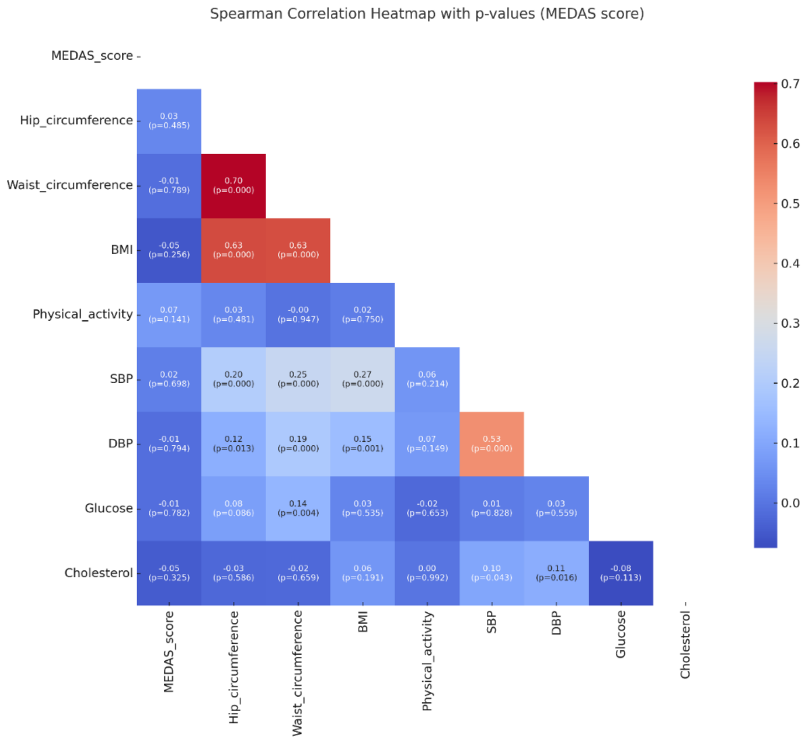

Figure 1. Correlation matrix of MEDAS score with antropometric values, blood preassure, glucose and cholesterol

Spearman correlation analysis revealed significant positive associations between BMI, waist circumference, and hip circumference, indicating interdependence among anthropometric measures. Both systolic and diastolic blood pressure were positively correlated with BMI and waist circumference. The MEDAS score (formerly labeled as the KIDMED index) showed no significant correlations with other variables. Physical activity demonstrated weak correlations with most variables, with no statistically significant associations observed.

Discussion

The results of our study enhance the understanding of how specific components of the Mediterranean and non-Mediterranean diets contribute to the prevention or risk of cardiovascular disease. Students from Split demonstrated better adherence to the Mediterranean diet compared to the other cities (Sarajevo and Mostar).

Regarding olive oil consumption, students from Split exhibited the highest frequency of olive oil use, as well as higher vegetable and fish consumption, which correlates with favorable laboratory and anthropometric parameters. Previous studies have shown that the incidence of major cardiovascular events is lower among individuals following a Mediterranean diet supplemented with extra-virgin olive oil or nuts, compared to those on a reduced-fat diet (Estruch, 2018).

The health science students from Sarajevo, a continental city, exhibited the highest statistical consumption of animal-derived fats (such as butter, cream, and margarine), the highest intake of sweetened beverages, and the lowest frequency of legume consumption. Students' diet in Sarajevo can be characterized as a hybrid of a partially Westernized diet and a traditional omnivorous dietary pattern.

While the average anthropometric and blood pressure values of students from Sarajevo were within normal ranges, the average cholesterol and triglyceride levels were elevated in women, suggesting a potential need for improvements in dietary habits and a reduction in cardiovascular risk factors.

Our analysis revealed significant positive associations between BMI, waist circumference, and hip circumference, indicating interdependence among anthropometric measures. Both systolic and diastolic blood pressure were positively correlated with BMI and waist circumference, suggesting a potential link between increased body mass and cardiovascular risk. The MEDAS score showed no significant correlations with other variables, implying a limited influence of adherence to the Mediterranean diet on the measured biometric and metabolic parameters in this sample. Physical activity demonstrated weak correlations with most variables, with no statistically significant associations observed.

An earlier study conducted on the student population in Rijeka, a city located on the Adriatic coast, revealed weak to moderate adherence to the Mediterranean diet (Pavičić Zezelj et al., 2019). In comparison, Sarajevo demonstrated even lower adherence to the Mediterranean diet, which may be partly attributed to its geographic location and the cultural differences influencing local dietary patterns.

Notable researchers in this field include Ancel Keys, a pioneer in investigating the Mediterranean diet and its impact on cardiovascular diseases, and Walter Willett and Frank Hu, who significantly advanced the understanding of the relationship between diet and chronic diseases (Hu & Willett, 2002; Aboul-Enein et al., 2017). Research on non-Mediterranean diets is less comprehensive, highlighting the need for further investigation in this area (Lugo-Morin & Bhat, 2022; Ghosh et al., 2023).

Previous research has demonstrated that high adherence to the Mediterranean diet is associated with a reduced incidence of all-cause mortality, fatal and non-fatal major cardiovascular diseases (CVD), type 2 diabetes, weight gain, metabolic syndrome, depression, cognitive decline, and nephrolithiasis. It is also linked to a lower average heart rate, mitigation of the harmful effects of overweight/obesity on CVD risk, and attenuation of the effects of obesity on type 2 diabetes. Additionally, there is evidence suggesting that the Mediterranean diet may enhance fertility (Carlos et al., 2017). The Mediterranean diet has consistently been shown to be a healthy dietary pattern in terms of morbidity and mortality (Sofi et al., 2013; Dinu et al., 2018), and it is widely recommended for the prevention of chronic and vascular diseases (Liyanage et al., 2016).

Previous studies have established a link between the consumption of animal fats and disturbances in lipid profiles (An et al., 2022). Additionally, a lower intake of legumes has been correlated with higher blood lipid levels (Zhang et al., 2010). Increased consumption of sweetened beverages has also been shown to elevate blood fat levels, posing a significant cardiovascular risk (Haslam et al., 2012).

Despite the well-documented health benefits of the Mediterranean diet, its adoption has been declining, even within countries around the Mediterranean Basin (Quarta et al., 2021). A global study on diet popularity ranked the Mediterranean diet 16th (Kamiński et al., 2020), which may be attributed to the indirect effects of social and cultural changes, increasing urbanization, and the globalization of food systems within Mediterranean countries (Bonaccio et al., 2014). This shift has resulted in a greater preference for Western-style diets, characterized by reduced fruit and vegetable consumption and an increased reliance on processed, convenient foods (Statovci et al., 2017). One potential solution to this unfavorable trend is a gradual return to Mediterranean dietary habits. This could involve promoting healthy lifestyle behaviors, such as increased consumption of fruits, vegetables, legumes, cereals, nuts, olive oil, and seafood, along with an emphasis on consuming fresh, local, and seasonal products. Furthermore, adopting regular moderate physical activity and promoting communal eating practices are essential components of a holistic approach to health.

On a global scale, research indicates that consumption of balanced diets and essential nutrients is often suboptimal. Significant gaps between current and optimal intake were observed for nuts and seeds, milk, and whole grains, with current consumption at only 12%, 16%, and 23%, respectively, of the recommended levels. Conversely, consumption of unbalanced diets, such as sugar-sweetened beverages and processed meats, significantly exceeds optimal levels, with global intake of processed meat exceeding optimal recommendations by 90% (Afshin et al., 2019).

Adopting a healthier lifestyle, while preserving cultural dietary traditions, is crucial to maximizing the health benefits of the Mediterranean diet, which has been shown to promote overall well-being and reduce the risk of chronic diseases (Bach-Faig et al., 2011). Additionally, the plant-based nature of the Mediterranean diet offers considerable environmental benefits, including reductions in greenhouse gas emissions (72%), land use (58%), and energy consumption (52%), along with a more moderate decrease in water consumption (33%). In contrast, adherence to a Western dietary pattern is linked to increases in these environmental impacts (Saez-Almendros et al., 2013).

Education on the importance of nutrition for cardiovascular health is essential for both students and the wider community. Implementing educational programs and workshops focused on healthy eating habits and their role in reducing the risk of cardiovascular diseases is critical. Previous studies have shown that lower adherence to the Mediterranean diet correlates with poorer health outcomes among students, including higher risks of depression. Alarmingly, many students, including those from medical and nutritional disciplines, demonstrate inadequate knowledge regarding healthy dietary practices (Antonopoulou et al., 2020).

Conclusion

The primary finding of our study is the low adherence to the Mediterranean diet among students, regardless of where they live. Their general health indicators are within the normal ranges and suggest no cardiovascular risk. Considering their age and the high proportion of students with low adherence to the Mediterranean diet, coupled with the observed dietary patterns, they may be at increased risk of health issues later in life, especially if they continue practicing the same lifestyle.

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Annex 1.

INDEX MEDAS- MEDAS questionnaire on Mediterranean nutrition (Mediterranean Diet Adherence Screener)

QUESTIONS	CRITERION FOR 1 POINT
Do you use olive oil as your main source? fat in cooking? (when preparing at least 2 meals a day, e.g. salads, cooked vegetables, meat or fish)	Yes
Which quantity olive oils use daily (including oil for salad, use during thermal processing of food, etc.)?	> 48 g
How much total serving vegetables you consume daily? (*1 serving = 1 cup fresh or cooked carrots, 1 cup cooked broccoli, spinach, 2 cups lettuce)	≥2
How much total serving fruit do you consume daily? (*1 serving = 1 piece fruit medium size, 1 cup strawberry, 1 banana)	≥3
How many total servings of red meat and/or meat products (dried meat, sausage and (fig.) you consume daily? (*1 serving = 100 - 150 g)	<1
How many total servings of butter, margarine or cream do you use daily? (*1 serving = 12 g or 1 tablespoon)	<1
How much carbonated and/or drinks with added sugar you consume daily?	<1
How much serving legumes (bean, peas, lens) you consume weekly? (*1 serving = 150 g)	≥ 3 servings
How many servings? fish and/or seafood you consume a week? (*1 serving = 100 - 150 g)	≥ 3 servings
How much times weekly you eat cakes and sweets?	< 3
How much total serving weekly you consume nuts fruits (almonds, walnuts, hazelnuts, peanuts)? (*1 serving = 30 g)	≥ 3 servings
You prefer whether rather consumption chicken, turkeys of red meat (veal, beef)?	That
How much times weekly you consume vegetables, pasta or rice?	≥ 2

Result: to 5 points indicates low compliance with MP (Mediterranean diet)., 6-9 indicates moderate compliance with MP
10 points and more indicates on high level adherence principles MP.

BIOACCESSIBILITY OF LYCOPENE: THE CRITICAL ROLE OF DIETARY FIBER INTERACTIONS IN DEVELOPMENT OF FOOD FOR SPECIAL MEDICAL PURPOSES

Dubravka Vitali Čepo[1], Nikolina Golub[1*], Kristina Radić[1], Emerik Galić[1], Marijana Ceilinger[2], Jelena Miličević[2], Lenkica Penava[2], Marijeta Kralj[3], Ivanka Jerić[3]

[1]University of Zagreb Faculty of Pharmacy and Biochemistry, Ante Kovačića 1, 10000 Zagreb, Croatia
[2]Belupo, Pharmaceuticals & Cosmetics Inc. Ulica Danica 5, 48000 Koprivnica, Croatia
[3]Ruđer Bošković Institute, Bijenička cesta 54, 10000 Zagreb, Croatia

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Summary

Lycopene is gaining recognition as a functional food component due to antioxidant and immunomodulatory properties that result with numerous health benefits. However, the interaction between lycopene and the food matrix, especially dietary fiber (DF), significantly impacts its bioaccessibility from food. This study investigates how different types of DF influence lycopene bioaccessibility, which is crucial for developing effective functional foods. The research utilized DF of different characteristics (inulin, oligofructose, dextrin, gummi arabicum, cellulose, pea fiber, apple fiber, and citrus fiber) to assess their lycopene binding capacity (LBC). Obtained results were further used to investigate if single fiber – LBC data can be used to predict LBC of DF mixture (by comparing experimental and calculated data). The study involved *in vitro* static simulation of gastrointestinal digestion to mimic gastric and intestinal conditions. The content and characteristics of DF were assessed by standard AOAC methods. Lycopene content was measured spectrophotometrically. Results showed that lycopene itself is stable under gastrointestinal conditions. However, the presence of different DF significantly altered lycopene bioaccessibility, that ranged from 50.7% to 111.3%, depending on the fiber type. The content of insoluble DF, higher oil holding capacity and shorter DF chain length corresponded with a lower lycopene bioaccessibility. Data on LBC of DF enabled relatively accurate prediction of LBC of DF mixtures. Understanding DF−lycopene interactions is crucial for formulating functional foods that maximize the health benefits of both lycopene and DFs. Further research should focus on the structural and physicochemical characteristics of DFs to optimize their combination with lycopene in food formulations, enhancing their bioavailability and health benefits.

Keywords: lycopene, dietary fiber, lycopene binding capacity, bioaccessibility, functional food

Introduction

Lycopene, a carotenoid found mainly in tomatoes and other red fruits, has become known for its significant health benefits, particularly in the area of medical foods. Its antioxidant properties are paramount, making it a component of interest in the prevention and treatment of various chronic diseases. Research indicates that lycopene can play a role in reducing the risk of certain cancers, particularly prostate and colorectal cancers, as well as mitigating metabolic disorders such as obesity and type 2 diabetes. Studies have shown that lycopene improves outcomes related to these metabolic diseases by alleviating oxidative stress and inflammation, which are significant contributors to their progression (Shafe et al., 2024; Kulawik et al., 2023). A recent review found that dietary lycopene intake (or serum lycopene levels) was inversely associated with all-cause mortality, prostate cancer, stroke, cardiovascular disease, metabolic syndrome, and male infertility (Li et al., 2021).

As the continuing research continues to uncover the potential health benefits of lycopene, it is increasingly being used in dietary supplements and functional foods to promote health and prevent chronic diseases. The global lycopene market is projected to reach approximately $196.6 million by 2030, growing at a compound annual growth rate (CAGR) of 4.9% from 2023 to 2030, reflecting a rising demand for natural antioxidants in the food and beverage sector. Furthermore, the increasing preference for ″clean label″ products has led manufacturers to favor natural ingredients like lycopene over synthetic alternatives. This shift is particularly evident in North America and Europe, where consumers are actively looking for foods that support health while avoiding artificial additives (Bothare, 2024).

Despite the growing body of evidence for the health benefits of lycopene, there remains a notable gap in understanding how exactly food matrix interacts with this carotenoid and affects its stability and bioaccessibility, and thus its bioavailability (Shahidi and Pan, 2022). This lack of information hinders the development of optimized formulation strategies that could enhance the bioavailability of lycopene from functional/special medical food formulations. Even

*Corresponding author: nikolina.golub@pharma.unizg.hr

though it is known that dietary fiber (DF), together with proteins, are the components of the food matrix that have the greatest impact on lycopene bioaccessibility (Molteni, 2022, 12.14.; Núñez-Gómez, 2023), the exact mechanisms of DF–lycopene interactions and the importance of particular DF characteristics on the ability to form complexes with lycopene have not been investigated so far. With the increasing need for foods for special medical purposes, which are usually rich in both DF and bioactive compounds such as carotenoids, there is a need to get better insight into structural- or physico-chemical characteristics of DF that are responsible for the occurrence of physiologically important interactions with bioactive compounds, in this case, lycopene. Therefore, the understanding physiologically relevant lycopene–DF interactions and the general acceptance of the bioaccessibility-based approach in functional food development is crucial for the formulation of functional foods that can maximize the health benefits of both DFs and lycopene (Xavier, and Mercadante, 2019). The main goal of this study is to investigate more thoroughly the significance of interactions of lycopene with DF/DF mixtures since it is essential for advancing our understanding and application of lycopene in dietary practices aimed at disease prevention and health promotion.

Materials and methods

DF (inulin, oligofructose, dextrin, gummi arabicum, cellulose, pea fiber, apple fiber and citrus fiber) utilized in this research were donated by Belupo Inc. (Koprivnica, Croatia). DF mixtures investigated for lycopene binding capacity (LBC) were designed by taking into account data on LBC for pure fibers and particular physico-chemical characteristics of pure fibers. The approximate composition of DF mixtures is presented in Table 1.

Table 1. Composition of dietary fiber mixtures

	IN/FOS /DEX+ CEL	IN/FOS /DEX +CEL[1]	MIX	MIX+ CEL	MIX*	MIX+ CEL*	SOL	SOL+ CIT	SOL+ CIT+ PEA
	Impact of increasing cellulose content		Impact of adding cellulose to the mixture/impact of the citrus DF particle size				Impact of adding citrus - and/or pea DF in the mixture		
FOS	+	+	+	+	+	+	+	+	+
Inulin	+	+	+	+	+	+	+	+	+
Dextrin	+	+	+	+	+	+	+	+	+
Cellulose II[1]	++	+	-	+	-	+	-	-	-
Gummi arabicum	-	-	+	+	+	+	+	+	+
Citrus fiber I [2]	-	-	+	+	-	-	-	+	+
Citrus fiber II [3]	-	-	-	-	+	+	-	-	-
Pea fiber	-	-	+	+	+	+	-	-	+

[1] fiber size 180 µm; [2]fiber size < 30 µm; [3]fiber size 75 µm. – fiber is not contained in the mixture

Lycopene used in experiments was Redvivo® from DSM (Limburg, Netherlands) – it is the formulation of lycopene uniformly dispersed in the modified starch matrix to ensure adequate water solubility. Total DF Assay Kit was from Megazyme® (Bray, Ireland). Bile salts, pancreatin from porcine pancreas (8 × USP) and lycopene ≥98% (HPLC) from tomato used for the preparation of calibration curve were from Sigma–Aldrich (St. Louis, MO, USA). Pepsin (from porcine gastric mucosa) 0.7 FIP-U/mg was from Merck (Darmstadt, Germany). All other chemicals were from Kemika (Zagreb, Croatia).

The amount of the total-, soluble- and insoluble- DF was determined using the Megazyme® procedure based on AOAC Method 991.43 “Total, Soluble, and Insoluble Dietary Fiber in Foods” (McCleary, 2023). It involves preparing an enzymatic digestion of the sample with heat-stable α-amylase at boiling temperature for 30 minutes, cooling the mixture, and treating it with protease at 60 °C for 30 minutes. After adjusting the pH, amyloglucosidase was added and incubated again. The mixture was then filtered to separate insoluble dietary fibre (IDF) from soluble dietary fibre (SDF), with SDF precipitated using ethanol. Finally, both fractions were dried and weighed to calculate the total dietary fibre (DF) content by summing the weights of IDF and SDF.

Water holding capacity (WHC) determination was based on the procedure of Robertson and Eastwood (1981) with some modification. Briefly, 50 mL Falcon tubes were labeled and weighed. 0.5 g of pure DF was placed into each Falcon tube, 20 mL of water was added to the fibers and the tubes were incubated at room temperature overnight. After incubation, the samples were centrifuged for 10 minutes at 4200 rpm to separate unbound water from the hydrated fibers. Following centrifugation, any remaining free water was carefully removed using an automatic pipette, ensuring that the sediment (hydrated fibers) remains undisturbed. Next, the Falcon tubes containing the hydrated fibers are weighed again to determine their final mass and the mass of bound water. WHC was calculated using formula [1].

$$WHC\ (\tfrac{g}{g}) = \frac{mass\ of\ bound\ water\ (g)}{mass\ of\ fibre\ (g)} \quad [1]$$

For the determination of oil holding capacity (OHC) modified procedure of Larrauri and co-workers (1996) was used, with some modification. Briefly, 15 mL Falcon tubes were weighed; 0.5 g of pure DF was added, following by the addition of 5 mL of sunflower oil. The tubes were incubated at 4 °C for 1 h and the samples were centrifuged for 15 minutes at 4200 rpm to separate any unabsorbed oil from the hydrated fibers. Following centrifugation, excess oil was carefully removed and the Falcon tubes containing the bound oil were weighed again to determine their final mass. OHC was calculated using formula [2].

$$OHC\ (\tfrac{g}{g}) = \frac{mass\ of\ bound\ oil\ (g)}{mass\ of\ fibre\ (g)} \quad [2]$$

Gastrointestinal stability of lycopene and DF binding capacity were obtained through an *in vitro* static simulation of gastrointestinal digestion, based on the standardized protocol established by Brodkorb et al. (2019), with some modifications. Initially, the samples were incubated in simulated gastric fluid (SGF) containing pepsin to mimic gastric conditions, maintained at 37 °C for 2.5 h in a water bath (Büchi B-490, Flawil, Switzerland) with consistent shaking at 110 rpm. Following this incubation, simulated intestinal fluid (SIF), which included bile salts and pancreatin, was added to the samples, and the reaction mixtures were incubated under identical conditions for another 2.5 h. Afterward, the samples were placed on ice for 10 minutes and then filtered through polypropylene hydrophilic membranes with a pore diameter of 20 µm to obtain clear filtrates suitable for the spectrophotometric determination of lycopene at 503 nm. Obtained absorbances were corrected by subtracting the absorbance of blanks containing SGF, SIF and enzymes. The concentration of lycopene was calculated based on the calibration curve of lycopene according to the equation [3].

$$y = 0.0044x - 0.0178 \quad [3]$$

where y is a lycopene concentration (mg/L), and x is the absorbance measured at 503 nm and corrected by the absorbance of blank.

For the preparation of lycopene−DF mixtures lycopene was dissolved in SGF in the concentration of 300 mg/L. 5 mL of this solution was added to 120 mg of pure fibers or 600 mg of the fiber mixtures in a 15 mL Falcon tube. The mixture was vortexed for 60 s to resuspend all the fibers. For the determination of lycopene gastric bioaccessibility (*t*=0 h) the mixture was centrifuged, and lycopene content was determined in the supernatant. For determination of gastric (*t*=2.5 h) or intestinal (*t*=5 h) bioaccessibility simulation of gastric or gastric and intestinal digestion was conducted as described above, prior to determination of lycopene content in the bioaccessible fraction.

In vitro bioaccessibility of lycopene from model mixtures was calculated according to the equation [4].

$$bioacessibility\ (\%) = \frac{bioaccesible\ lycopene}{total\ lycopene} x\ 100 \quad [4]$$

while total lycopene was determined after incubating the solution of lycopene (300 mg/L) in distilled water, in the dark, at room temperature, for 5 h. Theoretical bioaccessibility of lycopene form the fiber mixtures was calculated by multiplying the lycopene bioavailability from the model solution with a single DF with its ratio in the DF mixture and summarizing all obtained values.

Statistical analysis

DF content, WHC, OHC and lycopene bioaccessibility were analyzed in duplicates (n). DF–lycopene mixtures were prepared in duplicates, and each sample was assessed in triplicate for the lycopene content (n=6). Results are presented as means ± standard deviations. Obtained results were compared using Student's t-test or one-way analysis of variance and obtained differences were considered statistically significant when $p<0.05$. Software used for statistical analysis and graphical presentation of obtained results was Microsoft Excell (Microsoft Corporation, Washington, USA) and GraphPad Prism ver. 8.4.3 (GraphPad Software, Inc., CA, USA).

Results and discussion

The physico-chemical properties of food matrix can significantly influence the efficiency of digestion, especially by interfering with the interaction of the particular nutrient with digestive enzymes. The interaction required for successful absorption can only occur if the DF rich cell walls in plant tissues are broken down or the enzymes can diffuse through permeable cell walls. Since not all plant cell wall matrices behave the same during digestion, the highly permeable or easily disrupted cell walls enable greater bioaccessibility and earlier digestion of nutrients (Van den Bringen, 1999; Nunez-Gomez, 2023). On the other hand, free nutrients can be encapsulated by DF under the conditions of gastrointestinal tract which may hinder their incorporation into micelles necessary for absorption in the intestines but also, in some cases, protect them from the degradation in gastrointestinal tract.

As shown in Figure 1, lycopene used in this study proved to be stable under the conditions of the gastrointestinal system. The amount of bioaccessible lycopene changed from 94.9 ± 0.9 % at the beginning of gastric digestion up to 108.4 ± 8.9 % at the end of intestinal digestion with no significant difference between obtained results (p>0.05). This is not consistent with the available research on *in vitro* gastrointestinal stability of lycopene naturally present in food. Bilušić and co-workers (2019) showed significantly lower gastrointestinal stability of lycopene ranging from 59.8 ± 0.2 % (in carrot) up to 68.1 ± 0.1 % (red pepper). Similarly, Goni and co-workers (2006) showed that bioaccessibility of lycopene from vegetables is around 82% and from fruits around 33.3%. The authors assumed that low bioaccessibility is due to the presence of insoluble protein-lycopene and DF−lycopene complexes in the food matrix. Similar negative effect of DF on lycopene bioaccessibility has been confirmed by several scientific investigations *in vivo* (Riedl et al., 1999; van Het Hof, 2000). The main reason for higher bioaccessibility of lycopene in our study is the fact that lycopene naturally present in food exists in carotenoid-protein complexes or forms crystalline aggregates tightly bound to proteins that protect it from degradation but limit its release during digestion. In our study, free lycopene was added to the mixture of DF so its bioaccessibility could be reduced only in case of forming insoluble complexes during simulation of digestion or due to degradation under gastrointestinal conditions.

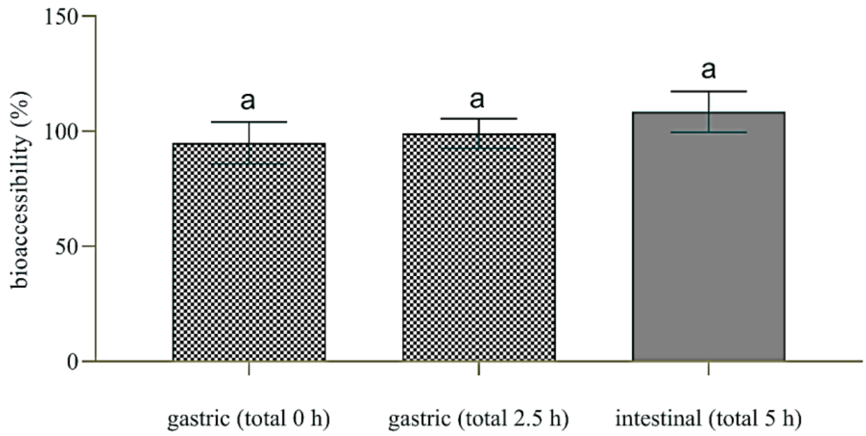

Fig. 1. Gastrointestinal bioaccessibility of lycopene
Columns marked with the same letters belong to the same statistical groups (p>0.05) as assessed by ANOVA and post hoc Tukey test.

Major characteristics (OHC, WHC, total-, soluble- and insoluble- DF content) of DF utilized in this investigation are presented in Table 2.

Table 2. Characteristics of pure dietary fiber analyzed for lycopene binding capacity

	OHC (g/g)	WHC (g/g)	TDF (g/100g)	SDF (g/100g)	IDF (g/100g)
fructooligosaccharides	0.8±0.06	-	88.5±4.2[4]	88.5±4.2[5]	-
inulin	1.1±0.02	1.5±0.15	95.2±7.1[4]	95.2±7.1[5]	-
dextrin	1.8±0.03	-	84.5±3.2	84.5±3.2	*
cellulose I[1]	3.1±0.04	5.8±0.47	99.0±6.5	0.66±0.05	98.3±4.3
cellulose II[2]	1.2±0.01	4.5±0.03	98.5±2.1	-	98.5±2.1
gummi arabicum	1.3±0.09	-	90±5.1	88.4±4.5	1.6±0.1
citrus fiber I[3]	1.2±0.09	8.2±0.45	70±4.3	31.9±0.1	38.8±4.1
citrus fiber II[4]	1.2±0.02	9.9±0.22	70±3.5	31.9±0.2	38.1±2.3
peas fiber	1.1±0.01	5.0±0.04	59.3±3.1	6.2±0.07	53.1±6.1
apple fiber	1.3±0.05	4.4±0.12	61.2±4.8	41.5±0.3	19.5±2.2

OHC-oil holding capacity; WHC-water holding capacity; TDF-total dietary fiber; SDF-soluble dietary fiber; IDF-insoluble dietary fiber:[1] fiber size 75 μm; [2] fiber size 180 μm; [3]fiber size < 30 μm; [4]fiber size 75 μm, [5]data obtained from the producer (since fructan-type fiber cannot be determined by the dietary fiber determination method applied)-not determined.

The results presented show that commercially available DF differ significantly regarding their purity i.e., TDF content. Obtained values ranged from 59.3% (in peas fiber) up to 99.0% in cellulose fiber. Four types of analyzed DF were predominantly soluble (FOS, inulin, dextrin and gummi arabicum); two types were predominantly insoluble (two types of cellulose, with different chain lengths) and four types of DF were mixed type, containing both soluble and insoluble fraction, in different ratios (two types of citrus fiber, peas fiber and apple fiber).
Results presented in Table 2 also show that there are significant differences in OHC and particularly WHC among tested DF, as well. WHC and OHC are fundamental characteristics that influence the physiological effects of DF, impacting digestion, nutrient absorption, and overall gut health. WHC allows DFs to retain water, which is essential for increasing stool bulk and promoting regular bowel movements. Limited literature data indicate that DF with high WHC can also trap nutrients, including water-soluble vitamins and minerals, thereby affecting their absorption. The OHC is important for its ability to bind fats and fat-soluble nutrients which might modulate the absorption of liposoluble compounds from food. WHC values ranged from 1.5 ± 0.2 g/g or inulin, up to 9.9 ± 0.2 g/g for citrus fiber II. OHC ranged from 0.8 ± 0.1 g/g in FOS up to 3.1 ± 0.0 for cellulose I. Obtained values are consistent with available literature data (Jiang 2022, Wagner 2024, data obtained from the manufacturer).

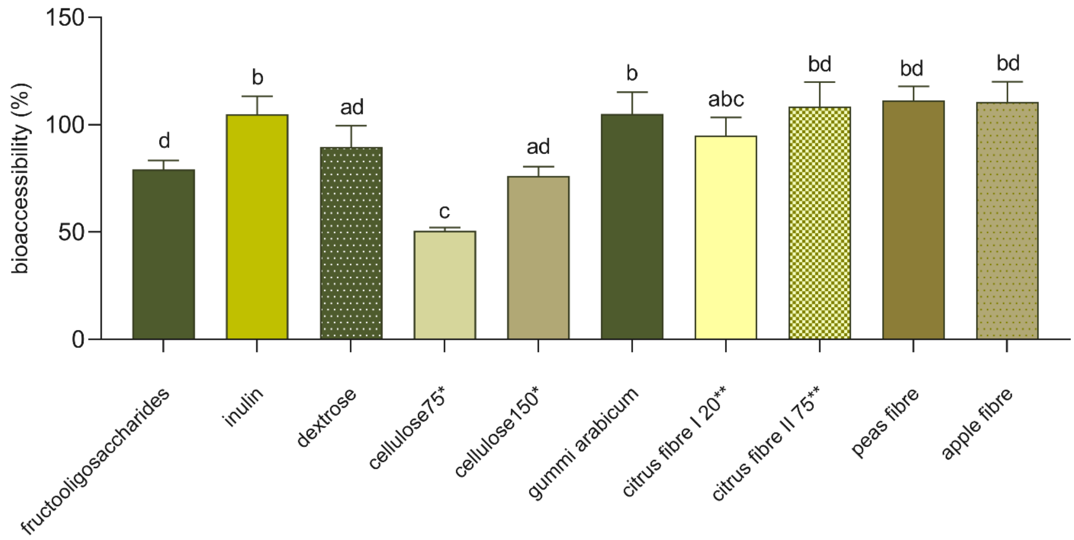

Fig. 2. *In vitro* bioaccessibility of lycopene from model mixtures with dietary fiber
Columns marked with different letters belong to different statistical groups ($p<0.05$). *Approximate length of dietary fiber; **average mesh size. cellulose I-fiber size 75 μm; cellulose II-fiber size 180 μm; citrus fiber I-fiber size < 30 μm; citrus fiber II-fiber size 75 μm.

Results presented in Figure 2 show that investigated DF differ significantly according to their LBC resulting in the wide range of lycopene bioaccessibility. Observed values ranged from 50.7 ± 1.5 % from the model solution containing cellulose I up to 108.4±11.5 % – 111.3±6.6 % in the model solutions containing citrus fiber II and peas fiber, respectively. As presented in Table 3, the amount of TDF in the reaction mixture showed significant negative correlation with lycopene bioaccessibility (r=-0.6849; p=0.0289) and the effect was even more pronounced in the case of IDF (r=-0.8167; p=0.0250). The amount of SDF in the model solution seems to have no influence on the bioaccessibility of lycopene.

Table 3. Correlation between total dietary fiber (A), soluble dietary fiber (B), insoluble dietary fiber (C) and OHC (D) and lycopene bioaccessibility

	Correlation coefficient	correlation	Significance (p)
Total dietary fibre (g/100g)	-0.6849	moderate, negative	0.0289
Soluble dietary fibre (g/100g)	0.1910	no correlation	0.6625
Insoluble dietary fibre	-0.8167	strong, negative	0.025
OHC	-0.6800	moderate, negative	0.0305

WHC did not correlate with lycopene bioaccessibility from the model mixtures (r=0.0020; p=0.9966; data not shown), while higher OHC corresponded with a lower lycopene bioaccessibility. This is probably due to the more favorable formation of DF−lycopene complexes (Table 3). The data obtained also indicates that the length of the DF chain can also significantly influence formation of lycopene−DF complexes. The data of the LBC of pairs inulin - FOS and cellulose I - cellulose II clearly show that shorter chain length results in higher binding capacity. Namely, inulin is composed primarily of long-chain fructans with a degree of polymerization (DP) typically greater than 10 while FOS is a specific type of fructan that is shorter in chain length, with a DP ranging from 2 to 8 (data obtained from the producer). Similarly, in the case of cellulose, the average DF chain lengths of cellulose I and cellulose II are 75 µm and 180 µm, respectively, and this significantly influenced the binding of lycopene (Figure 2). The impact of particle size of the DF on lycopene bioaccessibility can be assessed by observing the differences in LBC of citrus fiber I (fiber size <30 µm) and citrus fiber II (fiber size 75 µm) that have the same chemical composition, but the citrus fiber II has slightly larger particle size. Our results show that the particle size of DF does not significantly influence lycopene bioacessibility since observed differences in lycopene bioacessibility from citrus I- and citrus II- mixtures were not statistically significant.

When trying to compare obtained data with the results of other studies, it becomes clear that these are very scarce and that most studies have been focused on fruits and vegetables that contain naturally present lycopene, leaving the exact insights into connections between DF structural characteristics and lycopene binding uninvestigated. Recent work of Feng and co-authors (2023) demonstrated the formation of complexes between lycopene and cellulose (as in our study) and showed that the cellulose network skeleton binds lycopene and improves its storage stability, thermal stability, and UV radiation stability. On the other hand, Gu and co-workers (2020) showed that in natural food matrices, SDF can bind significant amounts of lycopene. In their work, partial enzymatic degradation of SDF in tomato peel significantly improved extractability of lycopene. Similar observations were made by Li and co-workers (2022) who showed that the yield of lycopene from tomato pomace was raised by 57.2% after enzymatic treatment and partial degradation of DF.

In the formulation of foods for special medical purposes containing DF, the usual approach is to combine both SDF and IDF in adequate ratios. The DF composition is usually based on the recommendations for the intake of SDF and IDF for general nutrition or consistent with the guidelines for nutritional therapy of the particular health condition. Additionally, the impact of DF on textural and sensory characteristics of the product is also considered. In view of the above data, it would be useful to avoid the utilization of the DF with strong binding capacity for lycopene as it could negatively affect its bioaccessibility form the formulation.

However, it has not been investigated whether the data on LBC of the single DF can be used to approximate the LBC of the mixture of DF (with known DF composition). Namely, the synergistic or antagonistic effects of different DF within a mixture could lead to unexpected outcomes in the bioaccessibility of nutrients, indicating the need for comprehensive studies that consider the interactions within fiber mixtures rather than isolating individual components. Figure 3 shows lycopene bioaccessibility of experimental DF mixtures and compares obtained values with theoretically calculated based on LBC data obtained for single DF. Significant differences between obtained and expected (theoretically calculated) values indicate the possibility of synergistic or antagonistic interactions of DF.

Generally, bioaccessibility of lycopene from mixtures of DF was high, ranging from 71.8 ± 10.4 % in IN/FOS/DEX+CEL[1] mixture (containing the mixture of fructans and cellulose) up to 114.5±3.7 in MIX (containing the mixture of fructans and soluble DF). Comparison between experimentally determined lycopene bioaccessibility and the theoretical one shows that data on the interaction of lycopene with single DF can be used to predict lycopene bioaccessibility from the DF mixture. For example, the lowest lycopene bioaccessibility was assessed in the mixtures containing the highest amounts of cellulose (Table 1), and the effect of cellulose was higher in mixtures containing higher cellulose ratio (IN/FOS/DEX+CEL and IN/FOS/DEX+CEL[1]). However, it seems that the negative effects of IDF (such as cellulose) are often less pronounced in DF mixtures, as if the presence of SDF in the mixture decreases the possibility of IDF to bind lycopene. For example, the LBC of the DF mixture MIX (containing inulin, FOS, dextrin, gummi arabicum, citrus fiber and pea fiber) was lower than expected, indicating small but statistically significant solubility promoting effects of SDF. Also, the addition of cellulose to MIX decreased lycopene bioaccessibility to a lower extent, compared to theoretically determined level. Regardless of the observed differences, it can be concluded that data on the lycopene biding activity of the single DF can be useful in predicting the effects of DF mixture on lycopene bioaccessibility.

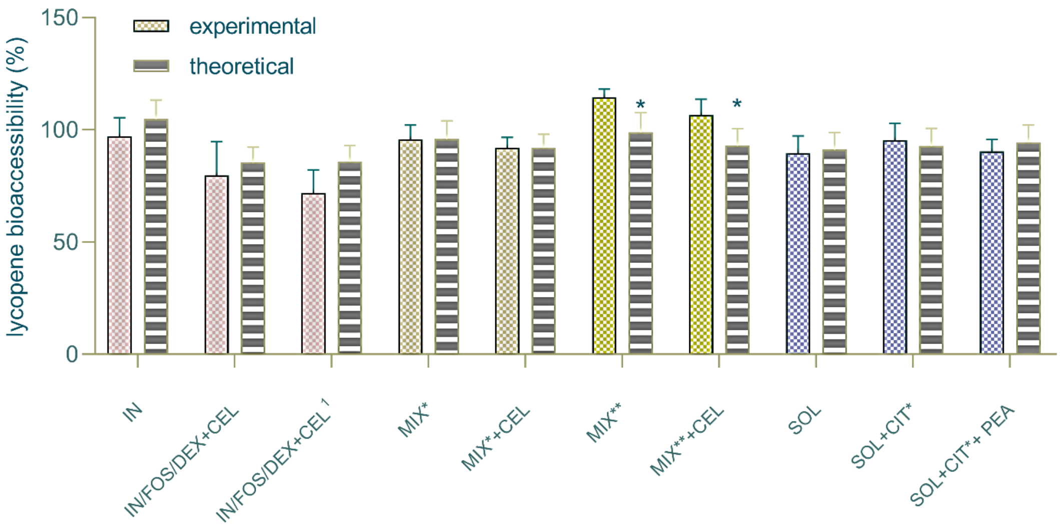

Fig. 3. Lycopene bioaccessibility from mixtures of dietary fiber (determined experimentally and calculated based on the composition of dietary fiber mixtures)

*Differences between experimental and theoretical lycopene bioaccessibility are statistically significant ($p<0.05$) indicating additive effects of dietary fiber in fiber mixture.

Conclusions

This study highlights the significant impact of DF and lycopene bioaccessibility, emphasizing the importance of understanding their interactions for the development of functional foods, particularly those intended for special medical purposes. The research demonstrates that different types of DFs bind lycopene to varying degrees, with SDF generally facilitating higher bioaccessibility compared to their insoluble counterparts. Obtained results show that the choice of DF in food formulations can be strategically optimized to enhance lycopene bioaccessibility, potentially offering opportunities for optimizing functional food formulations. However, additional research is required to confirm whether these effects lead to improved lycopene absorption and associated health benefits. Moreover, the study underscores the necessity for further investigation into the mechanisms governing DF−lycopene interactions. As the demand for functional foods continues to increase, a deeper understanding of these relationships will be essential to the development of effective dietary interventions to improve nutrient absorption and overall health outcomes.

Acknowledgment

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THE ROLE OF FUNCTIONAL FOODS IN MANAGING CHRONIC INFLAMMATORY CONDITIONS – A SYSTEMATIC REVIEW

Martina Arapović[1*], Leona Puljić[1], Nikolina Kajić[1], Sara Galić[1], Krešimir Mastanjević[2]

[1]University of Mostar, Faculty of Agriculture and Food Technology, Biskupa Čule bb, 88 000 Mostar, Bosnia and Herzegovina
[2]Josip Juraj Strossmayer University of Osijek, Faculty of Food Technology Osijek, Franje Kuhača 18, 31 000 Osijek, Croatia

review paper

Summary

Functional foods play an increasingly important role in the prevention and management of chronic inflammatory conditions due to their natural anti-inflammatory and antioxidant properties. Bioactive compounds such as polyphenols, flavonoids, omega-3 fatty acids, and probiotics have demonstrated positive effects in reducing inflammation and supporting immune function. Foods like turmeric, berries, green tea, garlic, and fatty fish have been linked to improvements in conditions such as rheumatoid arthritis, inflammatory bowel disease, and metabolic syndrome. The mechanisms behind these benefits include cytokine regulation, oxidative stress reduction, and modulation of the gut microbiota. However, while the current evidence is promising, it is mostly based on short-term or small-scale studies, with varying dosages and methodologies. Individual factors such as genetics and lifestyle also influence the effectiveness of these foods. Therefore, more robust, long-term clinical trials are needed to confirm their therapeutic potential and guide practical dietary recommendations. Overall, functional foods should not be seen as a replacement for conventional treatments, but rather as a complementary strategy. Future research should focus on personalized nutrition approaches and clearer guidelines to help integrate functional foods into routine healthcare and dietary practices.

Keywords: functional foods, chronic diseases, probiotics, prebiotics, functional peptides and proteins

Introduction

Chronic inflammatory diseases represent one of the leading public health challenges in modern society, with their prevalence continuously increasing (Bennett Jeanette et al., 2018). Low-grade inflammation is closely linked to the development of various diseases, including metabolic disorders, cardiovascular diseases, type 2 diabetes, neurodegenerative diseases, and certain types of cancer (Guarner and Rubio-Ruiz, 2015). The role of diet in modulating inflammatory processes has gained significant recognition, with functional food emerging as a potential factor in the prevention and management of chronic diseases. Functional food encompasses food products that, beyond their basic nutritional value, provide additional health benefits through components such as probiotics, prebiotics, dietary fibers, polyphenols, bioactive peptides and proteins (Vlaicu et al., 2023). An increasing number of studies are investigating their potential in modulating gut microbiota, regulating oxidative stress, and promoting anti-inflammatory responses. Therefore, the main goal of this literature review is to systematically explore and evaluate the role of functional foods in the prevention and management of chronic inflammatory diseases. The review aims to identify and analyze scientific evidence from recent human clinical trials that highlight the health benefits of various bioactive compounds found in functional foods, such as probiotics, prebiotics, dietary fibers, polyphenols, and functional proteins. By examining how these compounds influence inflammation, immune function, gut microbiota, and metabolic health, the review aims to better understand their therapeutic potential. Additionally, the objective is to provide insight into how functional foods can be integrated into modern dietary strategies as a complementary approach to traditional medical treatments, with the long-term goal of improving overall health outcomes and reducing the burden of chronic diseases. A literature search was conducted in the *PubMed/MEDLINE*, *ScienceDirect*, and *ResearchGate* databases using keywords related to functional food and chronic diseases. Studies published in the last five years (2020–2025) were included, provided they were open-access and represented randomized controlled trials (*RCTs*) conducted exclusively on human subjects. The study selection process was illustrated using a *PRISMA* flow diagram, and the final analysis included 37 studies that met the inclusion criteria.

Scientific Background on Functional Foods: Types and Importance

Functional foods are gaining increasing importance due to their ability to provide health benefits beyond basic nutrition (Ponte et al., 2025). These foods contain bioactive compounds such as probiotics, prebiotics, polyphenols, and peptides, which can help

*Corresponding author: martina.arapovic@aptf.sum.ba

prevent chronic diseases like cardiovascular disorders, diabetes, and obesity (Fernandes Lemos Junior et al., 2025). The demand for functional foods is driven by consumer awareness of the link between diet and health, along with advancements in food science that enable the development of nutrient-enriched products.

Categories of Functional Foods and Their Mechanisms of Action

The term "*functional foods*" refers to foods that enhance well-being, both mental and physical, and reduce the risk of disease, thereby promoting overall health (Mitsuoka, 2014). Therefore, they are categorized based on their health benefits and mechanisms of action (Figure 1).

Probiotics are live microorganisms, such as *Bifidobacterium* and *Lactobacillus* that improve gut microbiota composition, modulate the immune system, and produce antimicrobial substances to inhibit pathogenic bacteria (Arapović et al., 2024).

Prebiotics, including inulin and fructooligosaccharides, serve as food for probiotics, enhancing their survival and activity while increasing the production of short-chain fatty acids (*SCFA*s) that support gut health and reduce inflammation. (Markowiak and Śliżewska, 2017). Dietary fiber (*DF)* found in plant-based foods such as whole grains, fruits, vegetables, legumes, and nuts, plays a vital role in human health. It is categorized into soluble fibers (e.g., pectin in apples, β-glucan in oats) and insoluble fibers (e.g., cellulose in whole wheat, lignin in nuts), each offering distinct benefits. In digestion, *DF* enhances satiety, slows nutrient absorption, and promotes gut motility. Fermentable fibers like inulin (found in chicory and onions) and resistant starch (in green bananas and legumes) support gut microbiota by producing short-chain fatty acids (*SCFA*s), which improve metabolic and immune functions. (Yang et al., 2022). The study suggests that β-glucan significantly influences the adsorption of polyphenols from traditional apple varieties, highlighting its potential role in enhancing the bioactivity of these compounds (Jakobek et al., 2020).

Bioactive lipids, including omega-3 fatty acids, conjugated linoleic acid (*CLA*), and polyunsaturated fatty acids (*PUFA*s), play a crucial role in cardiovascular and brain health by reducing inflammation and oxidative stress (Mazzocchi et al., 2021).

Symbiotics, a combination of probiotics and prebiotics, work synergistically to enhance probiotic colonization, improve digestive and metabolic functions, and strengthen immune responses. (Markowiak and Śliżewska, 2017). Fermented foods such as yogurt, kefir, kimchi, and miso naturally contain probiotics that promote gut microbiota balance, increase nutrient bioavailability, and support metabolic health.

Functional proteins and peptides, derived from dairy, soy, or marine sources, offer additional benefits due to their antimicrobial, antioxidant, and anti-inflammatory properties, contributing to muscle function and cardiovascular health. Lastly, plant-based functional foods, such as berries, nuts, seeds, and whole grains, are rich in polyphenols, flavonoids, and dietary fibers, acting as antioxidants and promoting overall metabolic health.

Together, these functional food categories provide a diverse range of bioactive compounds that support human health through various physiological pathways. (Tarrah, 2022).

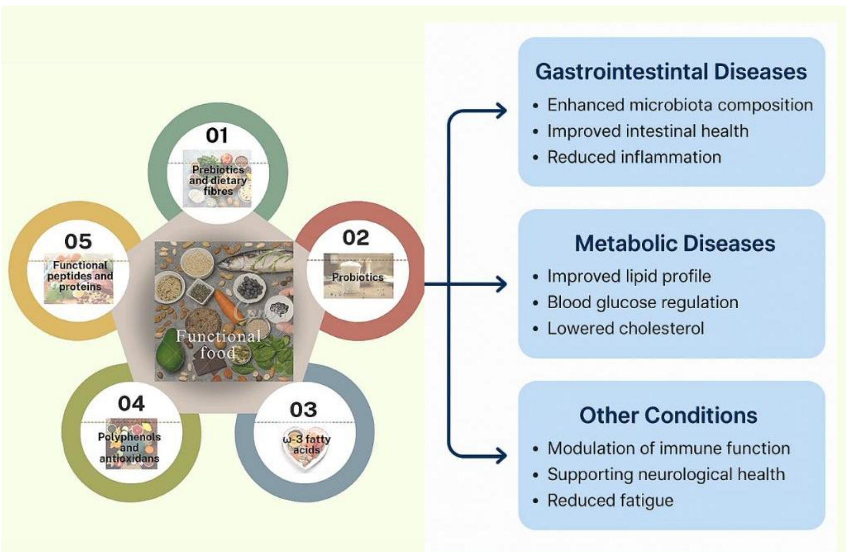

Figure 1. Categories of Functional Foods and health benefits

Materials and methods

A literature search was conducted in the *PubMed/MEDLINE, ScienceDirect* and *ResearchGate* databases using the following keywords: *functional food, chronic diseases, probiotics, prebiotics, dietary fibers, polyphenols, functional peptides, and proteins.* The inclusion criteria comprised studies published within the last five years (2020–2025), open-access availability, and randomized controlled trials (*RCTs*) conducted exclusively on human subjects. Exclusion criteria were review articles, studies without open access, and animal studies unless deemed relevant. The study selection process was carried out using the *PRISMA* flow diagram, which outlines the selection procedure. A total of 37 studies that met the inclusion criteria were analyzed (Figure 2).

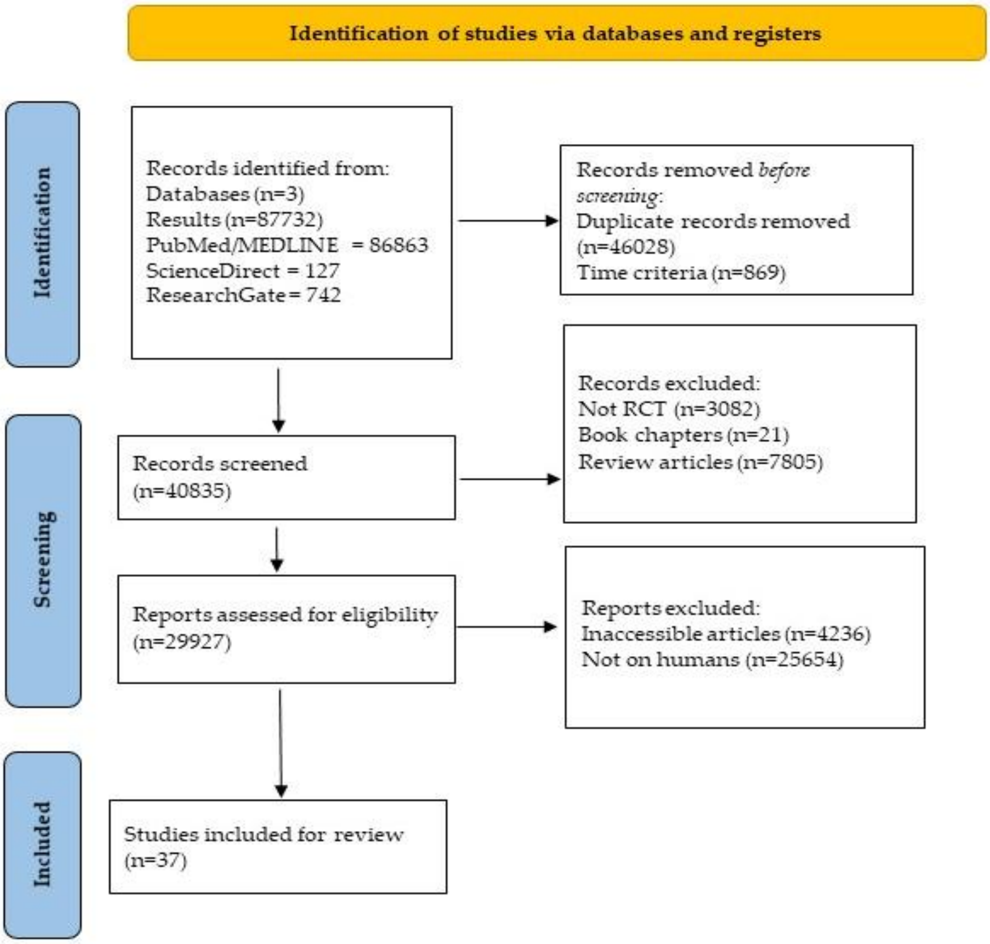

Figure 2. Utilization of the PRISMA flow diagram for conducting database searches and elucidating accessible articles for review

Results and discussion

Nutraceutical Effect of Functional Food Component

Prebiotics and probiotics play a crucial role in maintaining gut microbiota, which is essential for immune function and metabolic health. Vilander et al. (2022) investigated the roles of prebiotics in maintaining gut microbiota, which is crucial for immune function and metabolic health. They noted that rice is a major source of calories globally, and rice bran, a by-product of rice production, offers potential for further research. The food industry is continuously seeking innovative ways to utilize food by-products, and rice bran can be repurposed for health benefits. Their study found that rice bran supplementation increases gut microbiota diversity, enhances intestinal mucosal health, and elevates *IgA* levels in infants, indicating its immunomodulatory function (Table 1).

Table 1. Nutraceutical Effect of Functional Food Component

No.	Source	Type	Form	Findings	Reference
1.	Rice Bran	Prebiotic	Dietary Supplement	↑ gut microbiota diversity, improved gut mucosal health, elevated *sIgA* levels in infants	Vilander et al. (2022)
2.	*GI* Primer supplement	Prebiotic	Powder	Improved digestion, satiety, and overall well-being	Nekrasov et al. (2024)
3.	7 strain probiotic	Probiotic	Four seven-strain probiotic capsules (10^{10} *CFU*)	Probiotics could reduce inflammation and improve *GCS* in severe traumatic brain injury patients	Abbaszadeh et al. (2024)
4.	*L. plantarum*	Probiotic	Capsules	Treatment with probiotics showed clinical effectiveness in managing chronic diarrhea	Yang et al. (2021)
5.	*L.paracasei*	Probiotic	Capsules	*L. paracasei* capsules reduces *LDL*-C levels, oxidative stress, and inflammation in adults with high cholesterol	Khongrum et al. (2023)
6.	Beta-glucan	Fiber	80 g oat containing 3.0 g b-glucan	↓ *LDL* and total cholesterol, modulated gut microbiota, increased production of short-chain fatty acids	Xu et al. (2021)
7.	*DF* mixture	Fiber	10 g *DF* mixture	*DF* ameliorates renal anemia	Li et al. (2022)
8.	Inulin	Fiber	10 g/day of inulin	Dietary fiber supplementation may reduce inflammatory status in predialysis *CKD* patients in combination with low-protein diet	Chang et al. (2023)
9.	Resistant starch type 3 (*RS WM)*	Fiber	22 or 26 g/d for females and malesresistant starch type 3	Positively altered gut microbiota composition resulted in lower fasting glucose with no apparent change in appetite	Johnstone et al. (2020)
10.	Tapioca resistant maltodextrin (*TRM*)	Nutraceutical functional fiber	One serving (56 g) of *TRM*	Reduced HbA1C in both prediabetic and normoglycemic participants, decreased fasting plasma glucose in prediabetics, proved safe with no significant effects on liver and kidney function	Astina et al. (2022)
11.	*RPG** dietary fibre	Fiber	*RPG* powder, *RPG* capsules	*RPG* bread showing the best postprandial blood glucose-lowering effect, *RPG* dietary fiber powder and capsules improved satiety, reduced post-meal hunger, and increased serum *GLP-1*	Wu et al. (2023)
12.	Resistant maltodextrin (*RMD*)	Fiber	15 g or 25 g *RMD* crossover study	Effect of *RMD* on a specific microbial taxon that is potentially beneficial to human health, *F.saccharivorans*	Mai et al. (2022)
13.	Yeast beta-Glucan	Fiber	Four capsules (250 mg beta-glucan, 3.75 µg vitamin D_3, 1.05 mg vitamin B_6, and 7.5 mg zinc)	Yeast-derived beta-glucan may alleviate cognitive fatigue symptoms	Lacasa et al. (2023)
14.	ω-3 polyunsaturated fatty acid supplementation	ω-3 fatty acids	a high dose (3.5 g, n = 10) of *EPA* + *DHA*, a low dose (2.0 g, n = 10) of *EPA* + *DHA*, or placebo (olive oil, n = 12) via gel capsules	ω -3 *PUFA* supplementation improves protein homeostasis in *COPD* patients, with doses up to 3.5 g *EPA* and *DHA* being well tolerated and promoting protein gain	Engelen et al. (2022)
15.	Omega-3	ω-3 fatty acids	omega-3 (1.5 g of ω -3/day)	A diet supplemented with *EPA* and *DHA* is ideal for individuals with the *CC* genotype, as it provides direct products that bypass the affected synthesis step	Reyes-Pérez et al. (2024)
16.	Vitamin D3 and Marine Omega-3 Fatty Acids	ω-3 fatty acids	Supplementation	Vitamin D supplementation had a role in modulating the chronic inflammatory process, systemic inflammation, and possibly autoimmune disease progression	Dong et al. (2022)
17.	*Camu-camu*	Polyphenol	Capsules (1.5g/day)	Polyphenol-rich prebiotic may reduce liver fat in adults with overweight, reducing the risk of developing *NAFLD*	Agrinier et al. (2024)
18.	Resveratrol	Polyphenol	Capsules	*RSV* does not improve cardiometabolic risk factors, sympathetic activity, and endothelial function	Ferreira Gonçalinho et al. (2021)
19.		Polyphenol	2 capsules (480mg)	Helpful as an anti-inflammatory food supplement in chronic periodontitis patients	Nikniaz et al. (2023)
20.	Propolis	Polyphenol	Capsules (400 mg/day)	No significant reduction in uremic toxins, increase in gut microbiota richness and evenness	Fonseca et al. (2024)
21.	Beta- alanine	Functional peptides and proteins	supplementation (3.2 g/day)	Beta-alanine supplementation increased musclecar nosine in comparison with PL in patients with COPD (chronic obstructive pulmonary disease)	De Brandt et al. (2022)
22.	Casein	Functional peptides and proteins	20 g of whey protein or casein, diluted in 150 ml of water or juice	Acute supplementation with whey protein or casein similarly improves the HRQoL(health-related quality of life) of chronic liver disease patients	D'alessandro et al. (2021)

↑- increased value; ↓- decreased value; *sIgA*- total fecal secretory IgA; *GI*- gastrointestinal; *LDL*- low-density lipoprotein ; *DF*- dietary fiber; *GCS*- Glasgow Coma Scale; *CFU*- colony forming unit; *LDL-C*- low-density lipoprotein cholesterol; *EPA*- eicosapentaenoic acid; *DHA*- docosahexaenoic acid; *NAFLD*- non-alcoholic fatty liver disease; *PUFA*- polyunsaturated fatty acid; *COPD*- chronic obstructive pulmonary disease; *RSV*-; resveratrol; *RPG* - R+PolyGly dietary fiber products (bread, powder, and capsule); *CKD*- chronic kidney disease *; HbA1C*- hemoglobin A1C.

Nekrasov et al. (2024) conducted a study to investigate the effects of daily consumption of the *GI* primer supplement, which contains six functional blends, including prebiotics, fermented grasses, postbiotics, digestive enzymes, fruit and vegetable concentrates, spices, and select vitamins and minerals. Their findings showed that this supplement significantly improved digestive symptoms, stool consistency, energy, vitality, general health, and attitudes toward food and eating over a 14-day period. Notably, the positive effects began to emerge within just 1–2 days and persisted throughout the duration of the study. Furthermore, a seven-strain probiotic supplement has been shown to reduce inflammatory markers such as *IL-1β* and *TNF*-α while improving neurological outcomes in patients with severe traumatic brain injury (Abbaszadeh et al., 2024). Moreover, *Lactobacillus plantarum* and *Lactobacillus paracasei* have demonstrated potential in treating chronic diarrhea and lowering *LDL* cholesterol, contributing to atherosclerosis prevention (Yang et al., 2021; Khongrum et al., 2023). Dietary fibers exhibit a wide range of health effects, including lipid profile improvement, gut microbiota modulation, and blood glucose regulation. Oat-derived beta-glucan has been shown to reduce *LDL* and total cholesterol while promoting the production of short-chain fatty acids, highlighting its cardioprotective effect (Xu et al., 2021). Additionally, a dietary fiber blend has been found to improve anemia in patients with kidney disease (Li et al., 2022), whereas inulin reduces inflammatory markers in individuals with chronic kidney disease (Chang et al., 2023). Notably, resistant starch type 3 lowers fasting glucose and positively influences gut microbiota, suggesting its role in diabetes prevention (Johnstone et al., 2020). Similarly, the consumption of tapioca-resistant maltodextrin over 12 weeks resulted in reduced *HbA1c* (hemoglobin A1C) levels in individuals with prediabetes, further supporting its role in type 2 diabetes prevention (Astina et al., 2022). The study found that *RPG* (R+PolyGly -bread, powder, and capsule) dietary fiber improved the sensory properties of bread, enhanced satiety, reduced postprandial blood glucose, and increased serum insulin levels, with *RPG* capsules and powder showing the strongest appetite suppression and metabolic benefits, highlighting its potential as a functional food ingredient (Wu et al., 2023). Mai et al. (2022) in their study found that resistant maltodextrin (*RMD*) supplementation increased levels of *Fusicatenibacter saccharivorans* in healthy adults, with potential increases in *Akkermansia muciniphila* and *Faecalibacterium prausnitzii* observed in participants with low baseline levels, suggesting potential benefits for gut health that require further investigation. Finally, the study revealed that 36 weeks of yeast-derived beta-glucan supplementation significantly reduced cognitive fatigue in patients with *ME/CFS* (myalgic encephalomyelitis or chronic fatigue syndrome). This suggests it may be a beneficial nutritional supplement for alleviating cognitive dysfunction associated with this condition. However, further research is needed to understand the underlying mechanisms (Lacasa et al., 2023). Omega-3 fatty acids are essential for reducing systemic inflammation and metabolic syndrome. Omega-3 supplementation in patients with chronic obstructive pulmonary disease (*COPD*) has been found to improve protein homeostasis, potentially preventing muscle loss in this population (Engelen et al., 2022). Furthermore, omega-3 supplementation increased ω -3 fatty acids in red blood cells, but people with the *CC* genotype of the *FADS1 rs174547* variant showed a smaller increase compared to those with the TT genotype, confirming the variant affects fatty acid metabolism (Reyes-Perez et al., 2024). Additionally, vitamin D_3 and ω a-3 fatty acids have been associated with reduced chronic inflammation and the possible prevention of autoimmune diseases (Dong et al., 2022). Polyphenols are well known for their antioxidant and anti-inflammatory properties. Resveratrol, for example, improves lipid profiles and reduces systemic inflammation, while *camu-camu* exhibits hepatoprotective effects by reducing liver steatosis and regulating gut microbiota (Agrinier et al., 2024). Another study found that resveratrol increased total cholesterol and *apoB* but had no effect on plasma noradrenaline or endothelial function, suggesting it does not improve cardiometabolic risk factors or sympathetic activity (Gonçalinho et al., 2021). The study found that resveratrol supplementation significantly reduced plaque index (*PI*) in chronic periodontitis patients but did not significantly impact pocket depth (*PD*), clinical attachment loss (*CAL*), bleeding index, or inflammatory markers (*IL-8, IL-1β*), suggesting its potential as a complementary anti-inflammatory aid alongside standard periodontal treatments (Nikniaz et.al., 2023). Interestingly, propolis enhances gut microbiota diversity but does not significantly reduce uremic toxins in patients with kidney disease (Fonseca et al., 2024). Amino acid from functional peptides and proteins, beta-alanine has been shown to increase muscle carnosine levels by 54% in patients with *COPD;* however, it does not significantly improve exercise capacity (De Brandt et al., 2022). Casein and whey protein have demonstrated benefits in improving the quality of life in patients with chronic liver disease (D'Alessandro et al., 2021), further underscoring the importance of protein intake in maintaining metabolic health.

In summary, research shows that the food industry is exploring the repurposing of food byproducts like rice bran for potential health benefits. The *GI* primer supplement combines prebiotics, probiotics, digestive enzymes, and functional blends, improving digestive health, energy, and well-being within days. Key ingredients like beta-glucan, ω-3 fatty acids, and polyphenols support heart health, reduce inflammation, and regulate blood sugar. Dietary fibers such as oat-derived beta-glucan and resistant starch help lower cholesterol, enhance gut microbiota, and prevent diabetes. Additionally, probiotics like *Lactobacillus plantarum* and *Bifidobacterium longum* aid in reducing inflammation and supporting neurological health. These ingredients collectively offer wide-ranging benefits for metabolic, gastrointestinal, and cognitive health.

Functional Food Products for Managing Gut Microbiota and Chronic Diseases

Prebiotics such as inulin and agave fructans have been proven to influence the gut microbiota, improving digestion and the quality of life in patients with functional gastrointestinal disorders like irritable bowel syndrome (*IBS*). Studies have shown that the consumption of prebiotic snack bars enriched with inulin significantly increases the number of *Bifidobacterium* in the intestines (Reimer et al., 2020), while agave fructans in the form of jelly improve digestive function and psychological parameters in individuals with *IBS* (Camacho-Díaz et al., 2023) (Table 2). Although these results are promising, it is worth noting that much of the data comes from short-term or small-scale studies. Further large-scale trials are needed to validate these findings and assess long-term effects and safety.

Table 2. Functional Food Products for Managing Gut Microbiota and Chronic Diseases

No.	Source	Type	Enriched Product	Form	Findings	Reference
1.	Inulin	Prebiotic	Bars	Snack bar	Adding 3 or 7 g *ITF* to snack bars increased *Bifidobacterium* content	Reimer et al. (2020)
2.	Agave fructans	Prebiotic	Jelly	8g of jelly	Reduced anxiety, depression, enhanced quality of life in *IBS* constipation subtype patients, without laxative effects or intolerability	Camacho-Díaz et al. (2023)
3.	*Lacticaseibacillus paracasei* strain *Shirota* (LcS),	Probiotic	Milk	100 mL of a *LcS* beverage (108 *CFU*/mL)	Relieved constipation, improved potentially depressive symptoms, regulated intestinal microbiota associated with mental illness	Zhang et al. (2021)
4.	*L.gasseri*	Probiotic	Yogurt	85 g *LG21* strain-containing yogurt beverage	*LG21* strain have impact on mild to moderate delayed gastric emptying	Ohtsu et al. (2021)
5.	Cocoa	Polyphenol	Polyphenol-rich cocoa	Beverage (20 g cocoa powder)	↓postprandial *VLDL* and chylomicron particles, ↑*HDL-C*, reduced serum *IL-18* (inflammatory marker)	Davis et al. (2020)
6.	Fig	Polyphenol	Tea prepared from fig leaves	500 mL of fig leaf tea	Prolonged consumption of fig leaf tea may be a safe and effective alternative to current therapies for *AD* (atopic dermatitis)	Abe et al. (2022)
7.	*Thai mulberry*	Polyphenol	Tea	Concentrated beverage	*CMD* (concentrated mulberry drink) improved metabolic markers, particularly regarding its antihypertensive effects	Parklak et al. (2024)
8.	Mulberry leaf extract	Polyphenol	Tea	Beverage	Supplementation of *MLE* (Mulberry leaf extract) into real food could be practical and would potentially help to suppress postprandial blood glucose levels	Ding et al. (2023)
9.	Snack alternatives based on common beans	Fiber	Beans snack bar	32 g of the *CBBS*	The daily consumption of 32 g of a common bean baked snack (*CBBS*) reduces the blood levels of apolipoprotein *B-100*; this could positively influence cardiovascular health	Escobedo et al. (2021)
10.	β-glucan	Fiber	Whole grain barley	2 g, 4 g, and 6 g β-glucan	Postprandial glycemic response, high β-glucan whole grain barley foods could help to control blood glucose	Kellogg et al. (2025)

Table 3. Continued...

11.	β-glucan	Fiber	Oat product (*SoluOBC*)	3 g of β-Glucan (*OBG*) beverage	Reduce *SCI* and *CVD* risk in individuals with elevated baseline *LDL* cholesterol and *SCI*, suggesting that oats may benefit the inflammatory system and cardiovascular disease prevention in high-risk populations	Dioum et al. (2022)
12.	Barley dietary fiber	Fiber	Beverage	150 g of the beverage	*BDF* (barley dietary fiber) intake reduce hunger and increase satiety, may aid in managing postprandial glycemic responses and improving metabolic health	Kim et al. (2024)
13.	Gold Kiwifruit	Fiber	Gold Kiwifruit	2 pieces of kiwifruit	Effective as fiber-matched psyllium in treating constipation in adults, improved stool consistency, reduced straining	Bayer et al. (2022)
14.	Hemp seed protein (*HSP*)	. Functional peptides and proteins	Hemp seed	50 g casein/d, 50 g *HSP/d*, or 45 g *HSP* with an added 5 g of bioactive peptides (*HSPþ)*/d	Hemp protein consumption, as well as in combination with bioactive peptides, may have a role in the dietary management of hypertension	Samsamikor et al. (2024)
15.	The high protein investigational product (*IP*)	Functional peptides and proteins	Protein product	Ready-to-eat snack	Protein supplementation stimulated bacterial amino acid metabolism, protein supplementation alongside a mild energy restriction induces visceral fat mass loss and an activation of gut microbiota amino-acid metabolism	Kopecky et al. (2021)

ITF-inulin-type fructans; *IBS*- irritable bowel syndrome.; *VLD*L- very low density lipoprotein; *HDL-C*- high-density lipoprotein cholesterol, *IL-18*- interleukin-18, *CMD*- concentrated mulberry drink; *MLE*-; mulberry leaf extract *BDF*- barley dietary fiber; *SCI*- systemic chronic inflammation; *CVD* - cardiovascular disease; *HSP*- hemp seed protein.

Probiotic strains, such as *Lacticaseibacillus paracasei Shirota* and *Lactobacillus gasseri,* have also demonstrated the potential to modulate gut microbiota and alleviate symptoms of both constipation and depression (Zhang et al., 2021; Ohtsu et al., 2021). While the gut-brain axis represents an exciting frontier, current evidence largely relies on limited clinical trials, and more rigorous study designs are essential to determine strain-specific efficacy and optimal dosages.

Polyphenol-rich foods, including cocoa, fig leaf tea, and mulberry extracts, have been praised for their antioxidant and anti-inflammatory properties. Cocoa, for instance, has been shown to reduce postprandial *VLDL* and increase *HDL* levels, potentially supporting cardiovascular health (Davis et al., 2020). Meanwhile, fig leaf tea shows therapeutic promise for atopic dermatitis (Abe et al., 2022), and mulberry derivatives may aid glycemic control (Parklak et al., 2024; Ding et al., 2023). However, the bioavailability and mechanisms of action of polyphenols vary significantly across sources, raising questions about generalizability and clinical relevance of these outcomes.

Dietary fiber remains a cornerstone of functional nutrition, with a broad range of benefits supported by evidence. Fiber-rich snacks, such as those made from beans, may contribute to cardiovascular health by reducing apolipoprotein B-100 levels (Escobedo et al., 2021). Likewise, beta-glucan from oats and barley has shown cardiometabolic benefits, including reductions in postprandial glycemia and cholesterol levels (Kellogg et al., 2025; Dioum et al., 2022). Furthermore, satiety-enhancing fiber products, such as those used in bariatric formulations, appear to regulate appetite hormones like ghrelin and PYY (Kim et al., 2024). Even natural whole-food options like golden kiwis have been shown to rival psyllium in treating constipation (Bayer et al., 2022). These findings are encouraging, yet many studies lack long-term data and standardized interventions, which limits broader application.

Functional proteins, including hemp-derived proteins and those enriched with bioactive peptides, are gaining recognition for their potential roles in managing hypertension and improving metabolic health (Samsamikor et al., 2024). High-protein diets, particularly those implemented with mild energy restriction, have been linked to improved amino acid metabolism and reduced visceral fat (Kopecky et al., 2021). While these outcomes support the inclusion of protein-rich functional foods in metabolic interventions, their success is often context-dependent, varying with individual metabolic profiles and dietary patterns.

To sum up, research shows that probiotic strains like *Lacticaseibacillus paracasei* and *Lactobacillus gasseri* support gut health and improve digestion and mental health, alleviating symptoms of depression and constipation. Polyphenol-rich foods, including cocoa, fig leaf tea, and mulberry extracts, help reduce inflammation, improve metabolic parameters, and may aid in managing conditions like cardiovascular disease, diabetes, and atopic dermatitis. Fiber-rich foods, such as bean-based snacks, beta-glucan, and golden kiwis, enhance digestion, metabolism, and cardiovascular health, with benefits like reduced hunger and improved glycemic control. Protein products, including hemp-based proteins, aid in managing hypertension and support gut health, while high-protein diets can reduce visceral fat and improve amino acid metabolism.

Technological Challenges in Food Fortification with Bioactive Compounds – Innovations in the Food Industry

The fortification of foods with bioactive compounds presents several technological hurdles, primarily due to the instability and low bioavailability of these compounds. (Dahiya et al., 2023). Many bioactives, such as polyphenols, vitamins, probiotics, and omega-3 fatty acids, degrade easily when exposed to heat, light, oxygen, or certain pH levels, leading to a loss of their functional properties (Du et al., 2022). Additionally, some bioactives have poor solubility in water or fat, making their incorporation into various food matrices difficult without affecting sensory attributes like taste, texture, and color (Shahidi and Pan, 2021). To overcome these challenges, the food industry has adopted innovative strategies. Encapsulation technologies such as nanoencapsulation, microencapsulation, and liposomal delivery systems, help protect bioactives from environmental degradation and enhance their controlled release and absorption in the body (Pateiro et al., 2021). Banožić et al. (2025) explored the encapsulation of citrus aroma compounds from *Citrus reticulata* pomace using different coatings, such as gum arabic, maltodextrin, and carboxymethylcellulose, and found that freeze-drying effectively encapsulated volatile and semi-volatile compounds, with limonene, linalool, and α-terpineol being the most abundant aromas identified in disrupted microcapsules. Emulsification techniques, particularly nanoemulsions, improve the dispersion of lipophilic compounds, increasing their bioavailability. Furthermore, biopolymer-based carriers (such as alginate, chitosan, and protein-based matrices) provide structural stability to bioactive compounds, extending their shelf life (Gali et al., 2023). Recent advancements also include 3D food printing, which allows for precise placement and distribution of bioactives in food products, ensuring a consistent dosage (Mantihal et al., 2020). Additionally, the use of fermentation and enzymatic modifications has been explored to enhance the bioavailability of certain bioactives, making them more effective when consumed. These technological innovations are revolutionizing the functional food industry by improving the stability, efficiency, and sensory appeal of fortified foods, ensuring that consumers receive maximum health benefits without compromising on quality.

Conclusion

Functional foods possess significant potential in supporting the management of chronic inflammatory conditions. Their effectiveness lies primarily in the presence of bioactive compounds such as polyphenols, flavonoids, omega-3 fatty acids, and probiotics which exhibit anti-inflammatory, antioxidant and immunomodulatory properties. The integration of these foods into the daily diet shows promise in reducing systemic inflammation and improving clinical symptoms associated with diseases like rheumatoid arthritis, inflammatory bowel disease, and metabolic syndrome. However, despite these positive indications, the review highlights several limitations. Most available studies are short-term, involve small sample sizes, and often lack standardized methodologies. Variations in dosages, the bioavailability of active compounds and individual differences such as genetics and gut microbiota composition, also contribute to inconsistent outcomes. These factors underscore the need for more comprehensive, long-term clinical trials to fully validate the health benefits and therapeutic applications of functional foods. As a final point, functional foods should not be viewed as stand-alone treatments but as complementary elements within a broader, personalized dietary and medical strategy. Further interdisciplinary research is essential to develop clear, evidence-based guidelines for their effective use in managing chronic inflammation.

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THE ROLE OF QUERCETIN IN HUMAN HEALTH

Doris Drmač[1,2*], Martin Kondža[2,3]

[1]Pharmaceutical Health Institution LUPRIV PHARM, Rodočkih branitelja bb, 88000 Mostar, Bosnia and Herzegovina
[2]University of Mostar, Faculty of Pharmacy, Matice hrvatske bb, 88000 Mostar, Bosnia and Herzegovina
[3]Josip Juraj Strossmayer University of Osijek, Faculty of Food Technology Osijek,
Franje Kuhača 18, 31000 Osijek, Croatia

review paper

Summary

Medical plants have become more popular in the last decades due to their low price, natural origin, and fewer side effects. Researchers are attributing quercetin as one of the well-known types of plants metabolites. It is found naturally in many fruits, vegetables, flowers, bark, and leaves, but is not made in the human body. Food sources with a high content of quercetin include onions and citrus. This review article highlights quercetin's potential health benefits, anti-inflammatory and neuroprotective properties. Quercetin is widely used for its health-promoting properties. When taken as a dietary supplement or in food, it is used to improve various health conditions such as cardiovascular disease, allergies, diabetes, high blood pressure, cancer, and to help boost the immune system. Most studies look at the impact of flavonoids such as quercetin within the diet rather than as a supplement. Quercetin is also an antioxidant, which means it helps fight off free radicals that can cause oxidative stress. According to these data, quercetin could play a significant role in the exacerbation period of these diseases and have a therapeutic effect in treating the symptoms of the diseases.

Keywords: quercetin, cancer, diabetes, blood pressure, allergy

Introduction

In recent times, there has been a significant increase in the utilization of natural bioactives for managing chronic ailments owing to their low toxicity and environmentally friendly characteristics. Quercetin (Figure 1), a bioactive molecule, has been the subject of extensive research due to its diverse pharmacological activities, including antioxidant, neuroprotective, immune-modulatory, and anticancer activity with a low toxicity profile (Aghababaei et al., 2023). Quercetin (3,3′,4′,5,7-pentahydroxyflavone) is one of the most abundant dietary flavonoids and belongs to the flavonols subgroup. Flavonols (C6-C3-C6 polyphenols) have two hydroxylated benzene rings, A and B. Flavonols differ in the number and type of substitution in the B ring, where quercetin is dihydroxylated in positions 3′ and 4′.

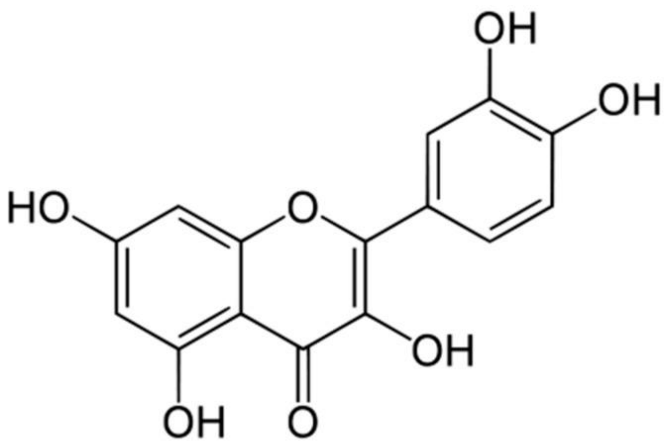

Figure 1. Chemical structure of quercetin

Quercetin is found in some fruits and vegetables, including onions, capers, apples, berries, tea, tomatoes, grapes, shallots, broccoli as well as pepper and many nuts, seeds, barks, flowers, and leaves (Wiczkowski et al., 2008). Quercetin has a broad pharmacological activity (Figure 2).

*Corresponding author: doris.drmac@farf.sum.ba

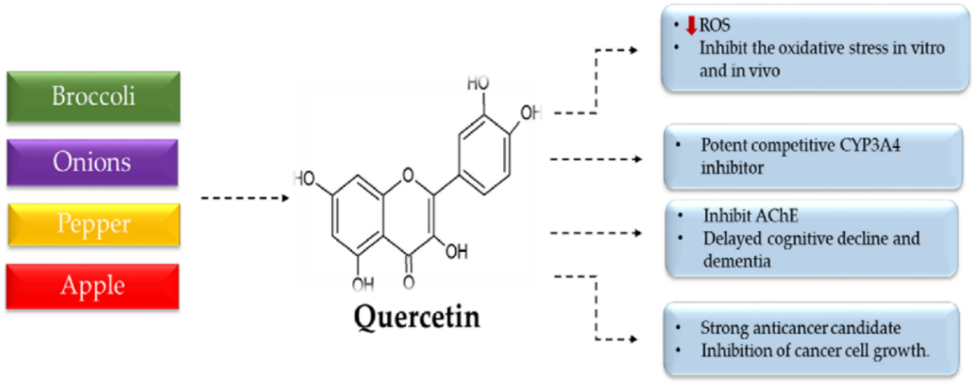

Figure 2. Pharmacological activity of quercetin (Salvamani et al., 2014)

Onions are thus qualitatively and quantitatively the most important source of quercetin. Among the investigated foods, it has been discovered that onions contain the highest amount of quercetin (approximately 300 mg/kg). According to the part of onions and shallots that is consumed, entirely various amounts and forms of quercetin are ingested. The quercetin forms of onion flesh include especially glucosides, with only minimal amounts of quercetin aglycone. Other vegetables, including broccoli, asparagus, green peppers, tomatoes, and red-leaf lettuce, could be great sources of ubiquitous quercetin, especially in the summer. Fruits (apples as well as berry crops, such as strawberries, red raspberries, blueberries, cranberries, and black currants), green tea, and wine could also be considered abundant dietary sources (Mlcek et al., 2016). In apples, another great quercetin source, there are well-studied antioxidant compounds such as quercetin-3-galactoside, quercetin-3-glucoside, and quercetin-3-arabinoside in the content range of 21–72 mg/kg; quercetin-3-rhamnoside; and quercetin-3-rutinoside. Quercetin conjugates are present entirely in the apple peels. Berry crops are rich in quercetin glycosides, with various types found across different berries. Strawberries and red raspberries contain quercetin-3-glucoside and quercetin-3-glucuronide, while these compounds, along with quercetin-3-rutinoside, are also present in blueberries. Additionally, quercetin-3-rhamnoside is found in red raspberries, black currants, blueberries, and cranberries, whereas quercetin-3-galactoside appears in blueberries, cranberries, and black currants, and quercetin-3-arabinose is specific to cranberries (Mlcek et al., 2016).

Flavonols present in red wine include aglycons, such as quercetin, myricetin, and kaempferol, as well as their glycosides (glucosides, galactosides, glucuronides and diglycosides). Quercetin is one of the most abundant flavonoids present in red wines. Among the flavonoid groups, these compounds are recognized as the main active compounds due to their wide range of biological activities (Castaldo et al., 2019).

The antioxidant and antimicrobial capacity of polyphenols has attracted increasing interest. The primary phenolic compounds found in tomato by-products are flavonols and phenolic acids with quercetin, naringenin, and rutin being the predominant molecules. These compounds have the ability to scavenge free radicals and reactive oxygen species, which are known to be involved in the development of cardiovascular diseases and several cancers.

Quercetin: chemical properties and health benefits

Quercetin is one of the most potent antioxidants of plant origin and is one of the predominant flavonoids found more commonly in edible plants. It belongs to the flavonols class of flavonoids, representing a major class of polyphenols. The dietary intake of total flavonoids is estimated to be 200–350 mg/day, and the intake of quercetin is 10–16 mg/day. The recommended dosage of quercetin aglycone as a dietary supplement is 1 g/day (the absorption is up to 60%) (Kim et al., 2019).

Quercetin is largely metabolized in the intestine and liver. The plasma level of quercetin is normally in low ranges, but after consuming foods that are highly rich in it, the plasma level increases to different ranges (Lakhanpal et al., 2007).

Quercetin is a potent molecule that can be used to cure various health-related issues. It is known to be used in the treatment of cancer, allergic reactions, inflammation, skin disorders and cardiovascular disorders (Batiha et al., 2020).

Experiments show that quercetin intake also has a positive impact in preventing and treating the occurrence of diabetes mellitus (Zu et al., 2021).
Even though quercetin has low bioavailability, it can pass through the blood-brain barrier (BBB) due to its lipophilic nature and functions as a neuroprotective. Administration of quercetin has been shown to enhance learning and memory performance while also reducing acetylcholinesterase (AChE) (Amanzadeh et al., 2019).
Quercetin is known for its antioxidant activity through free radical scavenging and its anti-allergic properties. These properties include immune system stimulation, antiviral activity, inhibition of histamine release, decrease in pro-inflammatory cytokines, leukotriene production, and especially suppression of interleukin IL-4 production, so it can help with diseases such as allergic asthma, allergic rhinitis (AR), and atopic dermatitis (AD) (Mlcek et al., 2016).

Neuroprotective effects

Neuroprotection by quercetin has been reported in several *in vitro* studies.
Quercetin has been shown to have anti-inflammatory properties and has been studied in various neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, and stroke. Some studies suggest that quercetin may have protective effects against neuroinflammation and potentially slow the progression of these diseases (Islam et al., 2021).

Alzheimer's disease

Alzheimer's disease (AD) contributes to 60–80% of total dementia cases, and it mostly affects elderly people (65 years of age or older). The pathogenesis of AD is typically associated with the accumulation of amyloid-β (Aβ) aggregates and the hyperphosphorylation of tau proteins, leading to neurofibrillary tangles (NFTs) and synaptic dysfunction. It has been shown that quercetin protects neurons from oxidative damage while reducing lipid peroxidation. In addition to its antioxidant properties, it inhibits the fibril formation of amyloid-β proteins, counteracting cell lyses and inflammatory cascade pathways (Khan et al., 2019).
Quercetin has also shown therapeutic efficacy, improving learning, memory, and cognitive functions in AD. Khan et al. (2009) and Shimmyo et al. (2008) concluded that quercetin administration resulted in the inhibition of AChE and secretase enzymes using *in vitro* models, thus preventing the degradation of acetylcholine, and decreasing Aβ production, respectively.

Parkinson's disease

Quercetin has been shown to have potentially beneficial effects on Parkinson's disease (PD) by targeting various mechanisms involved in the pathogenesis of the disease (Jung et al., 2018).
Here are some of the results and molecular mechanisms of quercetin in PD. Quercetin has been shown to have anti-inflammatory effects by inhibiting the production of pro-inflammatory cytokines and other inflammatory molecules. Chronic inflammation in the brain is believed to contribute to the development and progression of PD, and reducing inflammation may help slow down the disease (Table 1). Alpha-synuclein is a protein that forms aggregates in the brains of individuals with PD. Quercetin has been shown to inhibit alpha-synuclein aggregation and reduce the formation of these aggregates, which may help slow down the progression of the disease. It has been shown to have neuroprotective effects by protecting against neuronal damage and death in the brain. This may help slow the progression of PD and improve motor function (Chiang et al., 2023).
Inflammation plays a crucial role in the development and progression of a stroke, and reducing inflammation may help protect against neuronal damage. Guo et al. showed that quercetin has anti-inflammatory effects by inhibiting the production of pro-inflammatory cytokines and other inflammatory molecules in stroke (Guo et al., 2022).
These studies have shown that quercetin has potent anti-inflammatory and antioxidant properties, making it a potential candidate for neuroprotection and neuroinflammation.

Anti-allergic effects

Allergic rhinitis

Allergic rhinitis (AR) is a chronic disease with high prevalence worldwide, resulting in a huge health and economic burden. AR usually occurs in combination with asthma, conjunctivitis, and sinusitis (Bousquet et al., 2020).
The imbalance of innate and adaptive immunity is an important pathogenesis that induces AR, involving antigen-presenting cells, lymphocytes, and specific T cells. The imbalance between type 1 helper T (Th1) and Th2 cells is the immunological basis of AR. Th2 cells are the drivers of IgE-mediated allergic reactions that facilitate the release of IgE, IL-4, and IL-5. In addition, the dysfunction of regulatory T cells (Treg)/Th17 cells is also involved in the pathogenesis of AR (Table 1). Th17 cells regulate the pro-

inflammatory response, while Treg regulates the anti-inflammatory response (Ke et al., 2023).
Ke et al. (2023) considered quercetin to attenuate the inflammatory reactions of AR by improving the imbalance of Th1/Th2 and Treg/Th17 and inhibiting the increase of inflammatory cells, including epithelial cells, eosinophils, neutrophils, lymphocytes, and macrophages. They established the AR mice model and used multiple differentiations of quercetin to treat it. First, the nasal symptoms were assessed. The results illustrated that quercetin reduced the number of rubbing and sneezing in mice in a dose-dependent way.

Asthma

Asthma is a disease of the airways with various clinical and pathophysiological features, such as increased mucus secretion, reversible bronchial obstruction, airway hyperresponsiveness and narrowing, goblet cell hyperplasia, and inflammation. About 315 million people worldwide have asthma. This rate increases by 50% every decade and negatively affects public health (Huang et al., 2020).
$CD4^+$ T cells play a central role in mediating immune responses in allergic asthma. T helper (Th) 1 and Th17 cells contribute to the recruitment of neutrophils and are linked to more severe forms of asthma that may not respond to steroids. Th9 cells play roles in stimulating mucus secretion, attracting mast cells, and enhancing IgE levels. Other immune cells like $CD8^+$ T cells, NKT cells and gamma delta T cells also influence inflammation and airway hyperresponsiveness (AHR) in asthma. On the other hand, regulatory T (Treg) cells work to reduce both innate and adaptive immune activity, thereby lowering inflammation. While research has expanded our understanding of various T cell subsets in asthma, Th2-type immune responses remain the most closely tied to the asthma's underlying mechanisms (Lloyd et al., 2010).
In 2017, the effects of several herbs were investigated by Luo et al. (2018). The study shows that quercetin could be used to develop new bronchodilators to treat obstructive lung diseases such as asthma and chronic obstructive pulmonary disease. Different effects of quercetin, such as inhibitory effect on mast cell activation, eosinophil activation, relaxation of tracheal ring, reduction in IL-4 and IgE serum, blocking airway epithelial cell IL-8 and MCP-1 expression, suggest a valuable role of quercetin for allergic asthma. It is also suggested that quercetin might be a therapeutic candidate for allergic asthma and provide new insight into the immunopharmacological role of quercetin.
Oliveira et al. (2015) investigated the effect of onion extract and quercetin on cytokines and on smooth muscle contraction *in vitro* and its effectiveness in a murine model of asthma. After a treatment with onion extract or quercetin, they examined a decrease in pro-inflammatory cytokines creation, a release of tracheal rings, and a lowering of cell quantity in bronchoalveolar lavage and eosinophil peroxidase in the lungs.
As the plant-derived flavonoid quercetin is part of many foods and seems to be safe despite long-term use in animals and humans, therefore, its microemulsion would form an interesting and practical formulation to increase its oral bioavailability and, in turn, to evaluate its potential clinical advantage for treating certain inflammatory and allergic diseases (Rogerio et al., 2010).

Skin benefits

Because of its beneficial health effects and the fact that it is easily available from plants and food industry byproducts, quercetin has the potential to be used in medicine, particularly in the treatment of skin diseases.
The presented studies focus on the role of quercetin in the prevention and treatment of dermatological diseases analyzing its effect at a molecular level, its signal transduction, and metabolism. Aspects of quercetin's potential for skin treatment include protection against anti-aging and UV radiation, wound healing stimulation, and therapeutic effects on atopic dermatitis (Zaborowski et al., 2024).

Atopic dermatitis

The number of atopic dermatitis (AD) patients has been increasing steadily worldwide. AD is a dangerous disorder because it not only causes chronic inflammation but also leads to bacterial and viral skin infections. Quercetin has a therapeutic effect on AD through suppression of angiogenesis and Th2-related cytokine expression, including TSLP and TARC, in an AD-like Nc/Nga mouse model. Jung et at. suggest that quercetin might be an effective and improved therapeutic strategy that should be investigated further for the treatment of AD (Jung et al., 2010).
The results of testing the anti-inflammatory effects of quercetin in a mouse model showed that quercetin reduced the severity of dermatitis, mast cell infiltration, and epidermis thickness. Quecetin can also reduce the expression levels of TNF-α, CCL17, CCL22, IFN-γ, IL-4, and IL-6 (Hou et al., 2019).

UV radiation

When it comes to UV radiation, quercetin prevents the degradation of collagen due to UV radiation in human skin and inhibits MMP-1 and COX-2 expression. Quercetin inhibits UV-induced AP-1 activity and NF-κB. Additionally, quercetin may reduce phosphorylation of ERK, JNK, and AKT, and STAT3. Kinase assays using purified protein demonstrated quercetin's ability to directly inhibit the activity of PKCδ and JAK2. This suggests its possible direct interaction with PKCδ and JAK2 in the skin, counteracting UV-induced aging (Shin et al., 2019).
Research by Vicentini et al. (2011) confirmed reduction in skin irritation due to UV radiation by inhibition of NF-κB and such inflammatory cytokines as IL-1β, IL-6, IL-8, and TNF-α.
Kim et al. (2020) point to the fact that quercetin in propolis reduces PDK-1 and AKT phosphorylation, which suggests its efficacy in preventing UV-induced photoaging. Furthermore, when combined with caffeic acid ester and apigenin, quercetin reduces PI3K activity, further enhancing its protective effect.
Conversely, studies by Chondrogianni et al. (2010) on quercetin and its derivative, quercetin caprylate, showed that both compounds induce changes in the physiological characteristics of cells, including a localized whitening effect.

Wound healing

Wound healing is a natural restorative response that involves cell migration in tissue damage. Quercetin can accelerate wound healing by downregulating pro-inflammatory cytokines and supporting antioxidant capacity. The mechanism of quercetin impact on the improvement in wound healing quality was investigated by Baken et al. (2020). They performed a cell migration assay to investigate the effect of quercetin on tissue repair. AD-stimulating agents significantly delayed wound closure compared to the control group, indicating that tissue repair is impaired in AD. On the contrary, the single application of quercetin or its coadministration with AD-inducing agents was significantly effective in closing the wound gap.
Gopalakrishnan et al. (2016) also emphasized the significant role of TGF-β1 and VEGF activation by quercetin in the wound healing acceleration process. Two groups of rats: a control group and a Que-treated group were subject to evaluation for 14 days. The quercetin-treated group exhibited a faster rate of wound closure compared to the control group. In addition to activating the above-mentioned compounds, quercetin significantly attenuated TNF-α activity, supported fibroblast proliferation processes and collagen activity, and induced IL-10 levels.

Cardiovascular protection

More and more studies have found that quercetin has great potential utilization value in cardiovascular diseases (CVD), such as antioxidant, antiplatelet aggregation, antibacterial, cholesterol lowering, endothelial cell protection, etc.
With the accelerating global population aging, the prevalence of hypertension in developing countries is also increasing each year, and most cases are of unknown etiology. Hypertension is a chronic disease associated with endothelial dysfunction, smooth muscle cell contraction, and hyperlipidemia.
Kim et al. (2018) found that quercetin can inhibit the contraction of vascular smooth muscle through AMPK signaling pathway, thereby playing a role in reducing blood pressure (Table 1).
Another randomized, double-blind, crossover clinical study found that 41 hypertensive patients had a significant decrease in blood pressure after 28 days of continuous administration of 730 mg quercetin (Edwards et al., 2007).
Quercetin (25mg/kg) can inhibit myocardial fibrosis by modulating the TGF-β/Smads pathway, thereby helping to treat arrhythmia (Wang et al., 2021).
There is also evidence that quercetin suppresses the progression of atherosclerosis in animal studies, and several anti-atherosclerotic mechanisms have been proposed (Garelnabi et al., 2014).

Diabetes mellitus

Natural substances are inexpensive and easily obtained. Therefore, they can be used as an alternative to treat diabetes and other pathologies. Quercetin, due to its antioxidant, anti-inflammatory, hypoglycemic, and hypolipidemic activities, is known to be involved in the treatment of type 2 diabetes mellitus. Quercetin reduces blood glucose levels and preserves both the function of islets cells and the number of β cells in model rats and mice with diabetes (Table 1). Experiments show that quercetin intake has a positive effect on preventing and treating diabetes mellitus (Hosseini et al., 2021).
Quercetin is regarded as a very important flavonoid with beneficiary metabolic functions. Studies performed by Mahabady et al. (2021) showed that the oral administration of 75 mg/kg of quercetin to diabetic rats reduced the number of placental glycogen cells as compared to the control group. The plant compound acts as an oxygen scavenger and is known to protect against lipid peroxidation when present in

circulation. The antioxidant property of quercetin prevents the *in vivo* and *in vitro* oxidation of biomolecules. Quercetin is known to prevent embryonic malformations in pregnant diabetic mice (Mahabady et al., 2021).

Cancer

Quercetin is a potent flavonoid known for its chemoprotective activities in various *in vivo* and *in vitro* models. The various anti-cancerous properties such as reduced proliferation, the ability for induction of apoptosis, inhibition of mitotic events, causing cell cycle arrest makes it a reliable molecule in the therapy for cancer. Quercetin can be used as a potent therapeutic but it has poor solubility, poor permeability, and low bioavailability.
Treatment with appropriate dose makes quercetin non-toxic and shows inhibitory effects on the formation of tumors. Various *in vivo* and *in vitro* studies show that quercetin promotes apoptosis, inhibits metastasis, and regulates the cell cycle. In colorectal cancer quercetin arrests the cell cycle, modulates receptors of estrogen, regulates signaling pathways, and hence exhibits its chemo-protective functions (Ullah et al., 2020).
It has been studied that in leukemia in the case of humans, quercetin arrests the cell cycle at G2. Quercetin is also known to regulate p53 related pathways in cancerous cells. It regulates the release of p53 and hence inhibits the activities of cyclin A, cyclin B, CDK2, and therefore stagnates the MCF-7 cells of breast cancer in the S phase of the cell cycle. Quercetin affects the apoptotic pathways of the cancerous cells and therefore induces the death of cancer cells (Table 1). Treatment with an appropriate dose of quercetin increases the apoptosis-inducing protein expression and reduces the expression of the apoptosis-inhibiting protein. Studies on human metastatic ovarian cancer PA-1 cell lines show that quercetin induces the apoptotic pathway that is mitochondrial-mediated and thus inhibits the growth of metastatic ovarian cancer cells (Yang et al., 2020).

Table 1. The various properties and the mechanism of action of quercetin

No.	Properties	Mechanism of Action	References
1	Neuroprotective effects	reduces neuronal oxidative damage and neuroinflammation by inhibiting the production of pro-inflammatory cytokines	Chiang et al. (2023)
2	Anti-allergic effects	improves the imbalance of Th1/Th2 and Treg/Th17 and inhibits the increase of epithelial cells, eosinophils, neutrophils, lymphocytes, and macrophages	Ke et al. (2023)
3	Skin benefits	reduces the expression levels of TNF-α, CCL17, CCL22, IFN-γ, IL-4, IL-6	Hou et al. (2019)
4	Cardiovascular protection	inhibits the contraction of vascular smooth muscle through the AMPK signaling pathway and modulates the TGF-β/Smads pathway	Kim et al. (2018) Wang et al. (2021)
5	Anti-diabetic effects	reduces the number of placental glycogen cells and protects against lipid peroxidation	Hosseini et al. (2021)
6	Anti-cancer effects	promotes apoptosis, inhibits metastasis and mitotic events causing cell arrest	Ullah et al. (2020)

Conclusion

Flavonoid polyphenols are most beneficial for down-regulating or suppressing inflammatory pathways and functions. Quercetin is considered one of the most widely distributed and well-known naturally derived flavonols, showing strong effects on immunity and inflammation mediated by leukocytes and other intracellular signals. Quercetin can play a significant role in different diseases. This flavonoid has various biological activities, which are mainly related to its ability to inhibit enzymes and its effects on immune responses. Quercetin is known to possess antioxidant properties and has a protective function against aging. It exerts a protective role against neurodegeneration. The molecule is known to lower blood glucose levels and preserve the function of β cells in diabetic rats and mice. It shows a positive impact on treating and preventing diabetes. Various *in vitro* and *in vivo* studies have shown that quercetin has anti-cancerous activities and can be a reliable drug in cancer therapy. It has been shown that quercetin has anti-inflammatory and neuroprotective effects, partly through its ability to modulate the activity of specific signaling pathways. All this explains why many experts recommend regularly consuming food sources that contain quercetin.

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THERAPEUTIC POTENTIAL OF THE PHYTOCHEMICAL COMPOSITION OF WILD POMEGRANATE JUICE (*Punica granatum* L.)

Nikolina Kajić[1*], Leona Puljić[1], Martina Arapović[1], Aleksandra Šupljeglav Jukić[2]

[1]University of Mostar, Faculty of Agriculture and Food Technology, Biskupa Čule bb, 88000 Mostar, Bosnia and Herzegovina
[2]University "Džemal Bijedić", Agro-Mediterranean Faculty, Sjeverni logor bb, 88000 Mostar, Bosnia and Herzegovina

review paper

Summary

Wild pomegranate (*Punica granatum* L.) is a fruit tree that grows in a wide range of subtropical and tropical geographic locations around the world. Wild pomegranate fruits are rich in nutritional and phytochemical components, and the most appreciated form of consumption is freshly squeezed juice. The chemical composition of wild pomegranate juice varies depending on the genotype, climatic conditions, degree of fruit ripeness and processing method. The juice is rich in organic acids and sugars that determine its organoleptic properties, while phenolic compounds, such as flavonoids, anthocyanins and ellagitannins, contribute to its bioactive properties. These phytochemical compounds are known for their antioxidant, anti-inflammatory, antimicrobial and potentially cardioprotective effects.

The therapeutic potential of wild pomegranate juice includes the prevention of oxidative stress, modulation of inflammatory processes, antibacterial activity and inhibition of cancer cell proliferation. The results of various studies indicate a significant possibility of using wild pomegranate in the prevention and treatment of various chronic diseases, including cardiovascular diseases and cancer.

This paper emphasizes the importance of further research aimed at optimizing the conditions for the production of wild pomegranate juice and the application of its bioactive properties in the medical and food industries.

Keywords: wild pomegranate juice, phytochemical composition, phenolic compounds, antioxidant effect, anticancer potential

Introduction

Wild pomegranate (*Punica granatum* L.) is geographically related to the Mediterranean and Iran, and is characterized by good adaptation to adverse environmental conditions (Eghbali et al., 2021). Wild pomegranate fruits are consumed fresh and processed, and freshly squeezed juice is the most preferred by consumers, due to its rich nutritional and phytochemical composition (Fahmy et al., 2020). The chemical composition of the juice is significantly influenced by the genotype, climatic conditions, and the degree of ripeness of the fruit at the time of harvest, as well as the method of juice preparation itself (Hegazi et al., 2021). The most common methods of juice preparation are cold pressing of whole fruits or only the edible part of the fruit (Mphahlele et al., 2016). The results of research on juice samples with regard to genotype, growing conditions, degree of ripeness, and method of preparation show significant differences in polyphenol concentrations, with special emphasis on differences in samples obtained from the whole fruit and the edible part (Topalović et al., 2021). The investigated wild pomegranate juice samples, regardless of the processing method, are characterized by high acidity, elevated electrical conductivity (EC) values, and a high proportion of soluble dry matter (Topalović et al., 2020). The composition of organic acids and sugars largely defines the organoleptic properties of wild pomegranate juice, including parameters such as pH, total titratable acidity, and sweetness levels (Ikegaya et al., 2019).

Wild pomegranate fruit is rich in bioactive compounds, which include flavonoids, phenolic acids, and vitamin C, contained in all parts of the fruit. It has been found to contain higher concentrations of total phenols and tannins compared to cultivated pomegranate varieties (Guo et al., 2022). Primary metabolites in pomegranate juice include sugars, organic acids, and vitamin C.

Numerous studies indicate that the consumption of foods rich in phenolic phytochemicals is significantly correlated with numerous beneficial effects on human health (Angelino et al., 2017). Phenolic phytochemicals are particularly well known for their antioxidant and antimicrobial activities, and are additionally attributed with anti-inflammatory, anti-allergic and anti-proliferative properties. The health effects of polyphenols largely depend on their concentration and antioxidant potential, which is subject to variation depending on genetic origin and agro-ecological conditions during ripening (Hulya et al., 2012).

Antioxidants are key components of the human diet, and there is growing interest in natural sources of antioxidants, especially from plants. Among the most important natural antioxidants are vitamins, such as vitamin E, vitamin C and β-carotene, and plant

*Corresponding author: nikolina.kajic@aptf.sum.ba

polyphenols. Their advantage is reflected in the ability to neutralize free radicals and reactive oxygen species, thereby contributing to the preservation of the nutritional value of food and reducing the risk of developing chronic diseases (Aloqbi et al., 2016). Antioxidants have diverse chemical structures and mechanisms of action, and the ability to inhibit or slow down the oxidation of free radicals and reactive oxygen species represents a key defense mechanism against oxidative stress. This process can cause damage to cellular lipids, DNA and proteins, which is considered one of the main causes of the development of chronic diseases, including cancer (Escarcega et al., 2020).

The aim of this work is to investigate and analyze the phytochemical composition of wild pomegranate juice (*Punica granatum* L.) and, through a comprehensive review of the scientific literature, evaluate its therapeutic potential by examining the biological activities of its constituents, with particular emphasis on antioxidant, anti-inflammatory, antimicrobial, and cardioprotective effects.

Chemical composition of wild pomegranate juice

Considering the growing interest in the functional components of wild pomegranate (*Punica granatum* L.), numerous studies have focused on understanding how various factors – from processing technology to geographical origin – influence its chemical composition and biological properties. The results of various studies indicate a high variability in the content and stability of primary and secondary metabolites of wild pomegranate, depending on the processing method, growing location, variety and part of the fruit analyzed (Bar-Ya'akov et al., 2019).

Primary metabolites

Water is the most abundant component of wild pomegranate juice, followed by soluble sugars, including sucrose, fructose and glucose. The content of total sugars and organic acids in wild pomegranate juice is subject to the influence of genotype, climatic conditions and the degree of fruit maturity. According to Li et al. (2015), pomegranate fruits grown in colder climates contain more glucose and fructose compared to those from warmer areas. As the fruit develops, a significant increase in the level of total soluble solids is recorded, which correlates with an increase in glucose and fructose concentrations, indicating a connection between the developmental stage of the pomegranate fruit and the process of sugar accumulation (Bar-Ya'akov et al., 2019).

Analysis of the content of organic acids in the edible part of the pomegranate fruit confirmed the presence of citric, malic, oxalic, succinic, tartaric and ascorbic acids. Among the identified acids, malic acid was the most abundant, while citric acid followed in concentration. Similar patterns of concentration distribution were recorded in all analyzed samples (Topalović et al., 2020).

Organic acid and sugar profiles represent key determinants of pomegranate fruit flavor (Nafeesa et al., 2020). Among organic acids, citric and malic acids are the most abundant, with the concentration of citric acid standing out as a reliable chemical indicator for differentiating varieties according to the sensory flavor profile (sweet, sour-sweet and sour). The conclusion of Peng's et al. (2025) research is the definition of fructose and citric acid as the main flavor components, and the variability of phenolic and volatile compounds of different varieties defines the potential for the selection of specific genotype intended for industrial processing.

Secondary metabolites

Phytochemicals, often referred to as bioactive substances, are a group of secondary plant metabolites that, although not essential nutrients, can have a significant biological effect on human health (Singh et al., 2023).

Results of research by Topalović et al. (2020) in wild pomegranate juice samples from Montenegro, a total of 97 phenolic compounds were determined. The presence of 23 anthocyanins and their derivatives, 33 ellagitannins and ellagic acid derivatives, 12 flavanols, 4 flavonol glycosides, 1 flavone, 17 hydroxybenzoic and 7 hydroxycinnamic acids and their derivatives was recorded. The highest concentrations were recorded for flavanols and ellagitannins and ellagic acid derivatives. Recent studies conducted on samples of commercial pomegranate juices indicate exceptionally high concentrations of bioactive phytochemicals, including flavan-3-ols, ellagitannins, hydroxybenzoic acids, and vitamin C (Topalović et al., 2021; Topalović et al., 2024). Comparative analysis of juice obtained from the whole fruit and only the edible part showed that juice from the whole fruit contains a higher concentration of total phenolic compounds and a lower pH value, which indicates a higher degree of bioactivity and greater functional value of such a preparation. The high content of antioxidant components, especially ellagitannins and punicalagins, further confirms the potential of wild pomegranate as a valuable functional food product. The presence of high-molecular condensed tannins further expands the understanding of phenolic variability, indicating polymerization processes that contribute to the stability of antioxidant capacity (El Moujahed et al., 2022). The results of the study by Escarceg et al. (2020) indicate the influence of geographical factors on the chemical composition of the pomegranate fruit, with different contents of phenols, flavonoids and antioxidants in different regions. This further emphasizes the importance of environmental and agronomic factors in the bioactive profile of pomegranate.

Pomegranate is a rich source of anthocyanins, which are responsible for the red color of the fruit. The dominant anthocyanins include cyanidin-3-O-glucoside, cyanidin-3,5-di-O-glucoside, delphinidin-3-O-glucoside, delphinidin-3,5-di-O-glucoside, pelargonidin-3-O-glucoside, and pelargonidin-3,5-di-O-glucoside. In addition to anthocyanins, the edible part of pomegranate also contains phenolic acids, among which *p*-coumaric acid, chlorogenic acid, ellagic acid and gallic acid stand out (Redha et al., 2018).

Research by Wu and Tian (2017) investigated the phytochemical diversity of pomegranate in detail, with special emphasis on anthocyanins and hydrolyzable tannins, such as punicalagin and ellagic acid, which are converted in the body into urolithins – secondary metabolites with pronounced biological effects. Legua et al. (2012) found a high anthocyanin content in Spanish pomegranate varieties, with cyanidin-3-glucoside playing a dominant role.

The results of research by Catania et al. (2020) showed that variations in applied pressure levels during the pressing process led to significant quantitative and qualitative changes in the concentrations of bioactive compounds. Increasing the pressure leads to an increase in the total content of polyphenols, while the anthocyanin concentration has the highest value at the beginning of the process, only to decrease significantly later. This change indicates a high sensitivity of anthocyanins to mechanical and oxidative processes, while volatile compounds show selective stability, whereby some compounds extract better, while others undergo degradation. This research highlights the importance of optimizing pressing conditions to preserve the functional and sensory properties of the juice.

A comparison of different methods of extracting juices from pomegranate fruit, including crushing and grinding of the edible part, whole fruit and halved fruit, resulted in higher concentrations of phenolic compounds and tannins in juices obtained from halved fruits, compared to other extraction methods (Mphahlele et al., 2016).

Industrial processing and type of packaging have a significant impact on the preservation of phenolic compounds in commercial juices. Research by Nilova et al. (2023) showed that directly squeezed juices have the highest antioxidant index, with tannins and total phenols playing a key role in the formation of bioactivity of juices.

Therapeutic potential of wild pomegranate juice

Wild pomegranate juice (*Punica granatum* L.) shows significant pharmacological properties, including antioxidant, anticancer, antiviral and antidiabetic activities, as well as a potential role in the prevention of cardiovascular diseases. The therapeutic effects of the extracts are primarily attributed to the presence and concentration of bioactive secondary metabolites, i.e. the high content of phenolic compounds, including anthocyanins, tannins, flavonoids and copigments (Guo et al., 2022).

A study by Aloqbi et al. (2016) was conducted to compare the antioxidant properties of standard punicalagin, the main polyphenolic compound present in pomegranate, and pomegranate juice, covering different mechanisms of antioxidant activity. In free radical scavenging tests, pomegranate juice was found to be more effective than standard punicalagin at higher concentrations. In contrast, in the reduction power test, an increase in activity was recorded with increasing concentration, with punicalagin being more effective than juice. The obtained results indicate the existence of different, but mutually complementary antioxidant mechanisms of action of punicalagin and pomegranate juice, which further supports their potential in the prevention of oxidative stress.

A solution of total pomegranate tannins significantly reduces the expression of a key mediator of the inflammatory response by 40.5% in an *ex vivo* model of human skin. Despite their high molecular weight, the extracts successfully penetrated the skin. The obtained results indicate the important role of tannin compounds, especially punicalagin, in the modulation of inflammatory processes, and support the potential of pomegranate peel extracts for topical application in the treatment of inflammatory skin conditions (Houston et al., 2017).

The results of the application of adsorptive membrane chromatography, as an advanced technique for the isolation of anthocyanin and copigment fractions from pomegranate juice extracts, indicate the synergistic effect of polyphenolic compounds, which is manifested in a significantly increased ability to neutralize free radicals and in a pronounced cytoprotective effect on cells exposed to oxidative stress (Kostka et al., 2020).

Pomegranate peel extracts, rich in polyphenolic compounds, further confirm their therapeutic potential, especially in the context of cardiovascular diseases. The use of a standardized hydroethanolic extract of pomegranate peel results in a reduction in plasma glucose and lipid levels, modulation of inflammatory processes, and stabilization of atherosclerotic plaques through an increase in collagen content and a reduction in the area of necrosis. In addition, the observed improvement in efferocytosis within atherosclerotic lesions suggests an additional mechanism by which pomegranate peel extract may reduce the progression and complications of atherosclerotic plaques, which has direct implications for the prevention of cardiovascular events. These findings are based on the study by Manickam et al. (2022). In addition to the pronounced cardioprotective and anti-inflammatory effects, the study by Daoutidou et al. (2021) further expands the spectrum of biological activity of pomegranate peel extracts by confirming their antibacterial potential. Efficacy was found against a wide range of pathogenic bacteria, including *Pseudomonas aeruginosa*, *Escherichia coli*, *Listeria monocytogenes*, *Salmonella spp.* and *Staphylococcus aureus*. These antimicrobial properties further highlight the potential for multiple applications of pomegranate peel extracts, especially within integrated therapeutic approaches aimed at the treatment and prevention of chronic non-communicable diseases with simultaneous antimicrobial protection. The results of the study indicate that wild pomegranate is a rich source of phytochemicals with potential medicinal applications, including quercetin, pyrogallol, malic acid and chlorogenic acid. These compounds may be responsible for the reported antibacterial activity and pronounced antioxidant potential against the bacterium *Xanthomonas oryzae* (Ullah et al., 2025).
In the work of Topalović et al. (2024), the *in vitro* cytotoxicity of wild pomegranate juices and their relationship with phytochemical composition were investigated. The analysis included different juice samples, identifying key bioactive compounds, including anthocyanins and ellagitannins, which act synergistically to inhibit the proliferation of cancer cells. The most pronounced cytotoxic effects were observed on lung and cervical cancer cells. The effects on breast cancer cells were less pronounced, and the lack of a significant effect of anthocyanin-rich juices on these cells suggests the importance of additional compounds, such as vitamin C and ellagic acid, for achieving the antitumor effect. Wild pomegranate juices did not show cytotoxicity towards normal cells, which highlights their potential as selective antitumor agents. The obtained results indicate that the combination and mutual ratios of phenolic compounds may be crucial for the targeted biological activity, rather than their total amount.
The research of Minutolo et al. (2023) is also significant, proving high antiproliferative effectiveness against breast cancer cells, without harmful effects on healthy cells, using hydrodynamic cavitation to extract phytocomplexes from whole pomegranate fruits.

Conclusion

Wild pomegranate juice (*Punica granatum* L.) shows significant therapeutic potential due to its rich phytochemical composition, which includes high concentrations of phenolic compounds such as anthocyanins, ellagitannins, flavonoids and vitamin C. These compounds possess pronounced biological properties, including antioxidant, anti-inflammatory, antimicrobial and cardioprotective effects, making wild pomegranate juice a valuable functional food product with potential for the prevention and therapy of various diseases, including cardiovascular diseases, cancer and infections. Also, research shows that different processing methods and growing conditions can significantly affect the concentration of bioactive compounds, highlighting the importance of optimizing production processes to preserve the functional properties of the juice. Wild pomegranate juice not only has the potential for use in the prevention and treatment of diseases, but also as a useful dietary supplement, due to its multiple health benefits. Due to its biological activity, wild pomegranate represents a source of valuable phytonutrients and can be considered important components of future therapeutic approaches.

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TRENUTNA SAZNANJA O UTJECAJU SUPLEMENTACIJE KREATIN MONOHIDRATA NA MOZAK I KOGNITIVNE SPOSOBNOSTI

Jozo Ištuk*

Sveučilište u Mostaru, Agronomski i prehrambeno-tehnološki fakultet, Biskupa Čule bb, 88 000 Mostar, Bosna i Hercegovina

stručni rad

Sažetak

Kreatin je organski spoj koji ima ključnu ulogu u energetskom metabolizmu, prvenstveno u mišićnom tkivu, no sve je više dokaza o njegovoj važnosti i u funkciji mozga. Kroz fosfokreatinski sustav, kreatin doprinosi održavanju stabilne razine adenozin trifosfata (ATP-a), osobito u uvjetima povećanih energetskih potreba poput mentalnog stresa, hipoksije ili starenja. Zbog toga je postavljena hipoteza da bi suplementacija kreatin monohidratom mogla imati pozitivan učinak na kognitivne funkcije. Cilj ovog rada bio je prikazati mehanizme djelovanja kreatina u mozgu te analizirati dostupne znanstvene dokaze o učincima suplementacije kreatinom na kognitivne sposobnosti kod odraslih osoba. Rezultati prikazuju da kreatin može imati pozitivan učinak na pamćenje, pažnju, radnu memoriju i brzinu obrade informacija. Najizraženiji učinci primijećeni su kod starijih osoba i populacije s potencijalno nižim razinama kreatina, poput vegetarijanaca. Učinak na izvršne funkcije i inteligenciju zasad je manje konzistentan. Zaključno, kreatin se pokazuje kao obećavajuća, relativno sigurna strategija za podršku kognitivnim funkcijama kod odraslih, no daljnja istraživanja su potrebna kako bi se potvrdila njegova učinkovitost i sigurnost u ovom području.

Ključne riječi: kreatin, suplementacija, mozak, kognitivne sposobnosti

Uvod

Kreatin je organski spoj koji igra ključnu ulogu u osiguravanju brze energije u tijelu. Primarno se nalazi u skeletnim mišićima, gdje kroz fosfokreatinski sustav omogućava regeneraciju adenozin trifosfata (ATP) – primarnog energetskog nositelja u stanicama (Wyss i Kaddurah-Daouk, 2000). Tradicionalno se kreatin doživljava kao dodatak prehrani koji podržava povećanje mišićne snage i izdržljivosti (Butts i sur., 2018), no njegova biološka važnost prelazi granice mišićnog metabolizma.

Kreatin možemo unijeti putem prehrane, osobito konzumacijom mesa i ribe (Brosnan i Brosnan, 2016), no značajan dio tijelu osigurava i endogena sinteza. Sinteza kreatina odvija se u jetri, bubrezima i gušterači, gdje se aminokiseline arginin, glicin i metionin koriste kao osnovni gradivni blokovi. Proces sinteze rezultira stvaranjem kreatina koji se potom transportira krvotokom do različitih tkiva, uključujući i mozak (Brosnan i sur., 2011). Ova endogena proizvodnja, međutim, može biti nedovoljna, osobito kod osoba s određenim prehrambenim obrascima (npr. vegetarijanci) ili u slučajevima genetskih poremećaja povezanih s kreatinskom biosintezom.

Važnost kreatina u mozgu potaknuta je otkrićem da, unatoč znatno nižoj koncentraciji u odnosu na mišiće, mozak koristi kreatin za održavanje energetskog metabolizma i podršku neuronalnim funkcijama. Prema nekim istraživanjima, kreatin posjeduje važnu ulogu u olakšavanju prijenosa energije unutar mozga, a prisutnost kreatin kinaze u središnjem živčanom sustavu ukazuje na njegovu funkcionalnu važnost (Godwin Elechi i sur., 2024). Nedostatak kreatina u mozgu, bilo zbog genetskih poremećaja poput guanidinoacetil metiltransferazne (GAMT) ili arginin:glicin amidinotransferazne (AGAT) deficijencije, povezan je s ozbiljnim neurološkim poremećajima i odgođenim neuro-razvojem kod djece (Braissant i sur., 2011). Ove genetski uvjetovane deficijencije pokazuju koliko je kreatin ključan za normalan razvoj i funkcioniranje mozga.

Danas se sve intenzivnije istražuje mogućnost da suplementacija kreatin monohidratom, koja je već dobro dokumentirana u kontekstu poboljšanja mišićnih performansi, može imati i pozitivan učinak na kognitivne sposobnosti (Godwin Elechi i sur., 2024). Preliminarni nalazi sugeriraju da, uz povećanu dostupnost energetskog supstrata, suplementacija može doprinijeti poboljšanju pamćenja, pažnje, brzine obrade informacija i opće mentalne funkcije, osobito u situacijama povećanih kognitivnih zahtjeva, mentalnog zamora ili smanjenog energetskog kapaciteta mozga (Forbes i sur., 2022). Posebna pažnja posvećena je populacijama s većim rizikom od niskih razina kreatina – primjerice, starijim osobama, vegetarijancima, te osobama s neurodegenerativnim stanjima – gdje bi suplementacija mogla igrati preventivnu ili terapeutsku ulogu (Forbes i sur., 2021).

S obzirom na sve navedeno, cilj ovog rada je pružiti detaljan uvid u mehanizam djelovanja kreatina u mozgu te dati sveobuhvatan pregled trenutne literature

*Corresponding author: jozo.istuk@aptf.sum.ba

o utjecaju suplementacije kreatin monohidratom na mozak i kognitivne sposobnosti kod odraslih.

Metode

Rad je temeljen na pregledu relevantne znanstvene i stručne literature, pri čemu su korišteni izvori s međunarodnih platformi kao što su PubMed, Google Scholar, Web of Science. U pretraživanju su korišteni ključni pojmovi i njihove kombinacije, poput: *kreatin*, *kreatin monohidrat*, *kognicija*, *mozak*, *pamćenje*, *neuroprotekcija*, *sigurnost* i *nuspojave*. Poseban naglasak stavljen je na studije koje istražuju učinke suplementacije kreatinom na kognitivne funkcije kod ljudi.

U obzir su uzeti znanstveni i stručni radovi, uključujući originalne istraživačke članke, pregledne radove i meta-analize. Literatura je selektirana prema relevantnosti za temu, a prednost su imala istraživanja provedena na ljudima i radovi koji koriste objektivne mjere kognitivnih funkcija. Radovi koji se isključivo bave sportskim performansama, ispitivanjima na životinjama ili korištenjem alternativnih oblika kreatina nisu bili primarno uključeni, osim u slučajevima kada su pružali dodatne mehanističke uvide.

Rezultati i rasprava

Metabolizam i transport kreatina u mozgu

Razina kreatina u mozgu ovisi o nekoliko čimbenika, uključujući endogenu sintezu, transport preko krvno-moždane barijere (KMB) i unutarstanični metabolizam. Endogena sinteza kreatina započinje u bubrezima, gdje se iz aminokiselina arginina i glicina formira guanidino acetatna kiselina. Ovaj prekursor zatim se transportira do jetre, gdje se metilira pomoću S-adenozilmetionina, čime nastaje kreatin (Wyss i Kaddurah-Daouk, 2000). Iako se smatralo da se sinteza kreatina prvenstveno odvija izvan mozga, enzimi potrebni za sintezu, arginin:glicin amidinotransferaza (AGAT) i gvanidinacetat N-metiltransferaza (GAMT), također su pronađeni u samom mozgu, što ukazuje na lokalnu proizvodnju, iako vjerojatno u manjoj mjeri (Allen, 2012).

Zbog prisutnosti KMB, koja strogo regulira prolaz tvari iz krvi u mozak, transport kreatina zahtijeva specifične mehanizme. Glavni put unosa kreatina u mozak je putem natrij-ovisnog i klorid-ovisnog kreatinskog transportera, poznatog kao SLC6A8. Ovaj transporter nalazi se na endotelnim stanicama kapilara koje čine KMB, kao i na neuronima i oligodendrocitima (Forbes i sur., 2022). Funkcionalnost ovog transportera ključna je za održavanje odgovarajućih razina kreatina u mozgu, a mutacije u genu SLC6A8 dovode do sindroma nedostatka kreatinskog transportera, karakteriziranog teškim neurološkim poremećajima (Farr i sur., 2020). Unutar moždanih stanica, kreatin prolazi kroz intracelularni metabolizam putem sustava kreatin kinaze/fosfokreatina (CK/PCr). Ovaj sustav uključuje enzim kreatin kinazu (CK), koji katalizira reverzibilnu reakciju prijenosa fosfatne skupine visoke energije između kreatina i adenozin trifosfata (ATP), čime se stvara fosfokreatin (PCr) i adenozin difosfat (ADP). Fosfokreatin služi kao brza rezerva energije, sposobna brzo regenerirati ATP iz ADP-a kada je energetska potražnja visoka (Hemmer i Walliman, 1993).

Uloga kreatina u bioenergetici mozga

Mozak je metabolički izuzetno aktivan organ koji troši otprilike 20 % ukupne tjelesne energije u mirovanju, unatoč tome što čini samo oko 2 % tjelesne mase (Allen, 2012). Održavanje energetske homeostaze ključno je za pravilno funkcioniranje neurona i drugih moždanih stanica. Kreatin igra vitalnu ulogu u ovom procesu putem fosfokreatinskog sustava, gdje fosfokreatin djeluje kao "skladište" fosfata za regeneraciju ATP-a. U mitohondrijima, gdje se ATP prvenstveno proizvodi putem oksidativne fosforilacije, mitohondrijska kreatin kinaza (uMt-CK) fosforilira kreatin u fosfokreatin koristeći novonastali ATP. Fosfokreatin zatim difundira kroz citosol do mjesta gdje je potrebna energija, poput ionskih pumpi na staničnoj membrani ili sinapsi. Tamo citosolna kreatin kinaza (npr., BB-CK) koristi fosfokreatin za brzu regeneraciju ATP-a iz ADP-a. Ovaj sustav osigurava da se razine ATP-a mogu brzo održavati, čak i tijekom perioda intenzivne neuronske aktivnosti (Rae i Bröer, 2015).

Sustav CK/PCr ključan je za održavanje energetske homeostaze mozga ne samo u normalnim uvjetima već i tijekom stresa. U stanjima poput hipoksije (nedostatka kisika) (Turner i sur., 2015), nedostatka sna ili intenzivne mentalne aktivnosti (Gordji-Nejad i sur., 2024), energetska potražnja mozga značajno se povećava. Kreatin pomaže u održavanju razine ATP-a u tim uvjetima, čime se štite neuroni od oštećenja uzrokovanih energetskim deficitom. S obzirom na navedeno postavljena je hipoteza da bi oralna suplementacija kreatin monohidratom – kroz povećanje razina fosfokreatina – mogla imati povoljan učinak na funkciju mozga. Ova pretpostavka potaknula je niz istraživanja koja su nastojala ispitati može li kreatin poboljšati kognitivne sposobnosti, ublažiti umor ili čak djelovati neuroprotektivno u različitim kliničkim i nekliničkim populacijama.

Nastavak rada je fokusiran na najnovije znanstvene dokaze koji ispituju učinke suplementacije kreatinom na kognitivne funkcije i mentalne izvedbe.

Učinci suplementacije kreatinom na kognitivne funkcije

Kako bi se integrirali dostupni podaci i procijenila stvarna učinkovitost suplementacije kreatin monohidratom na kognitivno zdravlje, provedeno je nekoliko sustavnih pregleda i meta-analiza (Avgerinos i sur., 2018; Prokopidis i sur., 2023; Xu i sur., 2024). U ovom radu bit će predstavljeni i uspoređeni nalazi triju ključnih pregleda literature, kao i nekih pojedinačnih istraživanja, koji su detaljno ispitali učinke suplementacije kreatina na različite domene kognitivne funkcije, uključujući pamćenje, pažnju i radnu memoriju, brzinu obrade podataka i ostale kognitivne funkcije.

a) Pamćenje

Metaanalize dosljedno ukazuju na pozitivan učinak suplementacije kreatinom na pamćenje. Prokopidis i sur. (2023) su kvantificirali ovaj učinak, otkrivši da kreatin poboljšava mjere pamćenja u usporedbi s placebom (SMD = 0,29, 95 % CI: 0,04-0,53). Ovaj rezultat sugerira malu, ali statistički značajnu korist od kreatina za pamćenje. Veličina učinka (SMD) od 0,29 ukazuje da je prosječno poboljšanje u skupini koja je uzimala kreatin bilo za 0,29 standardnih devijacija veće od poboljšanja u placebo skupini. Nadalje, autori su posebno istaknuli da je suplementacija kreatinom dovela do značajnog poboljšanja pamćenja u podskupini starijih odraslih osoba (SMD = 0,42, 95 % CI: 0,09-0,75). Ova veličina učinka je umjerena i ukazuje na izraženiji pozitivan učinak u ovoj dobnoj skupini. Xu i sur. (2024.) su također identificirali značajne pozitivne učinke suplementacije kreatinom na pamćenje kod starijih osoba. Ovi rezultati su važni jer pamćenje često opada s godinama, a kreatin bi mogao biti potencijalna intervencija za ublažavanje tog pada. Osim toga, sustavne analize su pokazale da kreatin ima pozitivan učinak na različite aspekte pamćenja (Avgerinos i sur., 2018). Suplementacija kreatinom je pozitivno utjecala i na verbalno pamćenje kroz testove „*prisjećanja popisa riječi*“ (Benton i Donohoe, 2011). Poboljšanja u ovim zadacima sugeriraju da kreatin može pomoći ljudima da efikasnije kodiraju, pohranjuju i dohvaćaju verbalne informacije.

b) Pažnja i radna memorija

U istraživanju koje su proveli Ling i sur. (2009) ispitani su kognitivni učinci suplementacije kreatin etil esterom, jednim od oblika kreatina, te je pružila uvide u njegov utjecaj na pažnju i radnu memoriju. Primijetili su smanjenje lažnih alarma u zadacima "*Arrow Flankers*", gdje sudionici moraju identificirati smjer središnje strelice, ignorirajući strelice koje ju okružuju. Poboljšanje u ovoj izvedbi uslijed suplementacije kreatinom ukazuje na poboljšanu selektivnu pažnju i kontrolu impulsa. Također su zabilježili poboljšanje broja točnih izostanaka u zadatku održane pažnje, što sugerira da kreatin može pomoći u održavanju budnosti i koncentracije tijekom dužih razdoblja.

Ling i sur. (2009) su također pronašli dokaze da kreatin poboljšava radnu memoriju, na primjer, u zadacima "*Number-Pair Matching*" (uparivanje brojeva). Radna memorija je ključna za privremeno pohranjivanje i manipuliranje informacijama, a poboljšanja u ovoj domeni mogu imati široke implikacije za svakodnevno funkcioniranje.

c) Brzina obrade podataka

Avgerinos i sur. (2018) su primijetili poboljšanja u vremenu reakcije u nekoliko zadataka, što sugerira da kreatin može ubrzati brzinu obrade informacija. Vrijeme reakcije, kao mjera brzine kojom pojedinac percipira i reagira na podražaj, ukazuje da kraće vrijeme reakcije odražava bržu brzinu obrade informacija. Poboljšanja u brzini obrade mogu imati značajne implikacije za svakodnevno funkcioniranje, olakšavajući brže donošenje odluka, poboljšavajući reflekse u situacijama koje zahtijevaju brze reakcije poput vožnje ili sporta, te povećavajući efikasnost u različitim zadacima, posebno onima koji zahtijevaju mentalnu agilnost.

d) Inteligencija

Za razliku od prethodnih metrika, utjecaj suplemetnacije kreatinom na inteligenciju je mjeren u samo nekoliko istraživanja (Ling i sur., 2009; Rea i sur. 2003; Rawson i sur., 2008). Dokazi su heterogeni – neka istraživanja vide benefite, dok druga ne. Važno je napomenuti da su ovo izolirani slučajevi i da je potrebno više istraživanja kako bi se potvrdio utjecaj suplementacije kreatinom na inteligenciju.

Važno je uzeti u obzir nekoliko razmatranja i ograničenja prilikom interpretacije nalaza o učincima kreatina na kognitivne funkcije. Metaanalize često kombiniraju studije koje se razlikuju u dizajnu, dozama kreatina, trajanju suplementacije i karakteristikama sudionika, što može utjecati na generalizabilnost rezultata i otežati usporedbu među studijama. Neki dokazi sugeriraju da bi specifične populacije, poput vegetarijanaca koji imaju niže početne razine kreatina, mogle imati veće koristi od suplementacije kreatinom za kogniciju (Benton i Donohoe, 2011), a starije odrasle osobe su identificirane kao populacija koja može imati značajne koristi od kreatina za pamćenje. Konačno, precizni mehanizmi kojima kreatin utječe na kogniciju još uvijek se istražuju, ali se vjeruje da uključuju poboljšanje energetske raspoloživosti u mozgu povećanjem razine fosfokreatina i neuroprotektivne učinke.

Sigurnost i preporučene doze

Kreatin monohidrat je, prema opsežnim istraživanjima, relativno siguran dodatak prehrani za zdrave pojedince, posebno u preporučenim dozama. Unatoč popularnim zabludama, kreatin ne uzrokuje dugotrajnu retenciju vode izvan mišića, nije anabolički steroid, ne uzrokuje oštećenje bubrega kod zdravih osoba, nema čvrstih dokaza da dovodi do gubitka kose, niti povećava rizik od dehidracije ili grčeva u mišićima. Kod nekih osoba, visoke doze mogu uzrokovati gastrointestinalne probleme (Antonio i sur., 2021).

Općenito, za suplementaciju kreatinom preporučuje se faza punjenja od 20 g/dan do 7 dana, nakon čega slijedi faza održavanja od 3-5 g/dan). Alternativno, može se koristiti dnevna doza od 0,10-0,14 g/kg tjelesne mase. Iako se ove preporuke prvenstveno odnose na povećanje mišićne mase i performanse, istraživanja također pokazuju blagotvorne učinke kreatina na funkciju mozga, a čini se da su opće smjernice doziranja primjenjive i kada se želi postići kognitivno poboljšanje (Candow i sur., 2024).

Zaključak

Kreatin, poznat kao dodatak za poboljšanje mišićne snage, sve više pokazuje važnu ulogu i u zdravlju mozga. Kroz fosfokreatinski sustav pomaže u održavanju stabilne razine ATP-a, osobito u uvjetima povećane mentalne aktivnosti, stresa ili starenja. Suplementacija kreatinom može imati pozitivne učinke na kognitivne funkcije, osobito pamćenje, pažnju i brzinu obrade informacija. Najveće koristi primijećene su kod starijih osoba i onih s potencijalno nižim razinama kreatina, poput vegetarijanaca.

Iako su potrebna dodatna istraživanja, trenutni dokazi sugeriraju da je kreatin sigurna, dostupna i potencijalno korisna strategija za podršku kognitivnom zdravlju kod odraslih.

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CURRENT KNOWLEDGE ON THE EFFECTS OF CREATINE MONOHYDRATE SUPPLEMENTATION ON THE BRAIN AND COGNITIVE ABILITIES

Jozo Ištuk

University of Mostar, Faculty of Agriculture and Food Technology, Biskupa Čule bb, 88 000 Mostar, Bosnia and Herzegovina

professional paper

Summary

Creatine is an organic compound that plays a key role in energy metabolism, primarily in muscle tissue, but increasing evidence highlights its importance in brain function as well. Through the phosphocreatine system, creatine helps maintain stable ATP levels, particularly under conditions of increased energy demand such as mental stress, hypoxia or aging. Consequently, the hypothesis has emerged that creatine monohydrate supplementation may have a positive effect on cognitive functions. The aim of this paper was to present the mechanisms of creatine action in the brain and to analyze the available scientific evidence on the effects of creatine supplementation on cognitive abilities in adults. The results indicate that creatine may positively influence memory, attention, working memory and information processing speed. The most pronounced effects have been observed in older adults and populations with potentially lower creatine levels, such as vegetarians. Effects on executive functions and intelligence are less consistent. In conclusion, creatine appears to be a promising and relatively safe strategy for supporting cognitive function in adults, but further research is needed to confirm its efficacy and safety in this context.

Keywords: creatine, supplementation, brain, cognitive function

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