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FOOD IN HEALTH AND DISEASE

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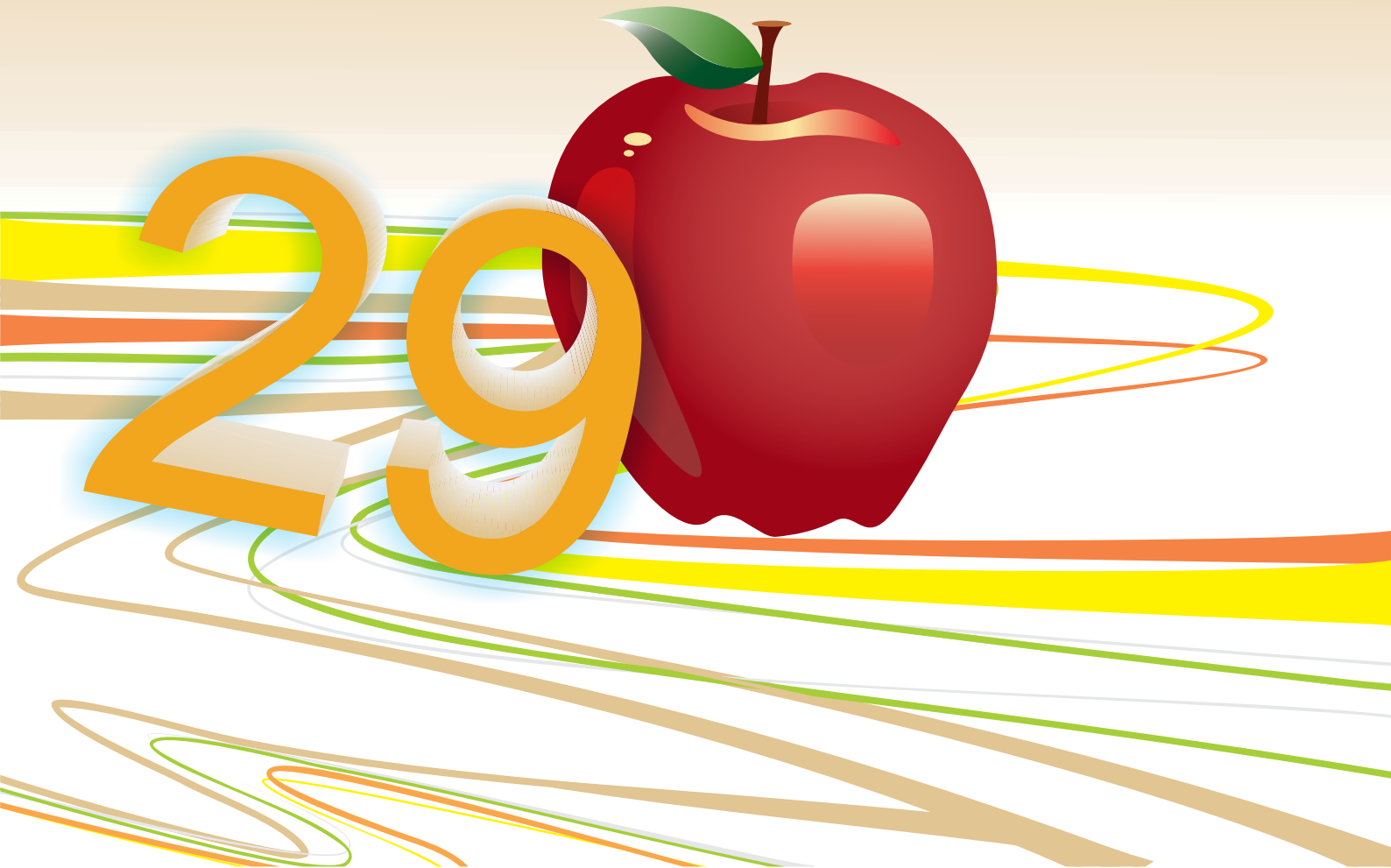

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BODY COMPOSITION, MUSCLE STRENGTH, DIET QUALITY AND EATING HABITS OF STUDENTS AT THE UNIVERSITY OF ZAGREB, CROATIA

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original scientific paper

Summary

Diet quality directly influences body composition, specifically the proportions of muscle, fat, and visceral fat. Elevated levels of body fat increase the risk of chronic, non-communicable diseases such as cardiovascular disease and type 2 diabetes. The Mediterranean diet is considered a sustainable model for preventing chronic diseases. This study aimed to assess the diet quality and body composition of 38 university students (63% female; mean age 24.4 ± 1.4 years) with varying levels of formal nutritional knowledge from four different faculties (social sciences, humanities, kinesiology, and nutrition) in Zagreb, Croatia. The Mediterranean diet served as the model for optimal diet quality. Data collected from May to September 2024 included anthropometric measurements (height, body weight and composition, and circumferences), three-day food diaries (analyzed using Prehrana software and the Mediterranean Diet Quality Index (MDQI)), a short questionnaire on health and dietary habits, and dominant handgrip strength testing (using Camry EH101 and Jamar Plus dynamometers). Students were generally adequately nourished (mean BMI: 23.2 kg/m²), with moderate muscle strength (*Camry*: 32.4 kg; *Jamar*: 33.2 kg), which was significantly higher in males (43.5 kg/44.4 kg) than in females (25.9 kg/26.6 kg). Average energy intake was 1869.8 ± 559.8 kcal, with a high percentage of fat (41%), a low percentage of carbohydrates (40.5%), and low fiber intake (7.7 g/1000 kcal). MDQI scores placed all students in the moderately poor adherence category (range: 8.7 to 10), characterized by high fat and low fruit and vegetable consumption, although Nutrition students demonstrated the best diet quality. Finally, the Camry EH101 proved to be a cost-effective alternative to the *Jamar* Plus dynamometer.

Keywords: body composition, muscle strength, dietary habits, students, MDQI

Introduction

Body composition is a key determinant of overall health, disease risk, and prognosis. High levels of body fat, particularly visceral adipose tissue, are associated with a significant risk of developing chronic non-communicable diseases such as type 2 diabetes, cardiovascular diseases, and non-alcoholic fatty liver disease (Finicelli et al., 2022). These risks are modifiable primarily through proper nutrition and regular physical activity, with overall diet quality having a greater impact than the consumption of isolated components. The Mediterranean diet (MD) is a well-established healthy dietary pattern typical of countries bordering the Mediterranean basin, characterized by a high daily intake of whole grains, nuts, low-fat dairy, and olive oil as the main fat source, along with moderate consumption of fish and poultry and infrequent consumption of red meat (Finicelli et al., 2022). Regular physical activity is an essential complement to this pattern, which has been recognized by UNESCO as intangible cultural heritage (Granic et al., 2019; Trichopoulou, 2021). The MD's protective mechanisms are attributed to its richness in dietary fiber, omega-3 fatty acids, and polyphenols, which together promote improved gut microbiota composition, reduce inflammation, enhance cardiovascular health by modulating lipid profiles, and increase insulin sensitivity. Adherence to the MD specifically correlates with greater muscle mass, muscle strength, and bone mineral density, providing substantial protection against conditions such as sarcopenia and osteoporosis (Granic et al., 2019; Jennings et al., 2020; Papadopulou et al., 2023; Andreo-López et al, 2023).

While proteins are essential for cellular function and the maintenance of muscle mass, long-term excessive intake (above 1.5 g/kg body weight) may pose a risk to renal function by inducing hyperfiltration and increasing metabolic byproducts such as urea (Martin et al., 2005), although this remains an area of ongoing research (Van Elswyk et al., 2018). High-protein, low-carbohydrate diets such as the ketogenic diet, while potentially beneficial for short-term reductions in glucose and triglycerides, often lead to unfavorable increases in LDL cholesterol and nutrient deficiencies (Levran et al., 2023; Budoff et al., 2024). Anthropometric parameters such as body mass index (BMI), waist-to-hip ratio (WHR), waist-to-height ratio (WHtR), and neck-to-height ratio (NHR) are reliable indicators of metabolic syndrome and other non-communicable diseases (Vaishya et al., 2024). Additionally, muscle strength, routinely measured by a hand-held dynamometer, is a crucial marker of

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overall muscle function and a strong predictor of health outcomes, especially in the aging population (Vaishya et al., 2024; Chai et al., 2024).
Non-invasive measurement of visceral adipose tissue, the endocrinologically active fat stored around internal organs, is typically performed using bioelectrical impedance analysis (BIA) and is essential for assessing metabolic risk. Optimal body composition and the prevention of associated diseases are closely linked to lifestyle choices. The aim of this study was to assess the diet quality of the student population, their adherence to the Mediterranean diet, and their body composition. Additionally, the objective was to evaluate the muscle strength of students using two different hand-held dynamometers (the analog *Jamar* and the digital *Camry*) to determine the acceptability and validity of these devices in the young adult population.

Materials and methodology

Participants

The study population included 38 students from the University of Zagreb, specifically selected from the Faculty of Economics (EFZG), Faculty of Law (PFZG), Faculty of Kinesiology (KIF), and Faculty of Food Technology and Biotechnology (PBF), due to differences in the formal knowledge about proper nutrition they receive during their studies. Participation was voluntary, with recruitment conducted by distributing an application form electronically through various online student platforms and social media. Data collection occurred over five months, from May to September 2024. In this study, basic anthropometric data and handgrip strength were measured for each student, followed by completion of a self-administered questionnaire on dietary and lifestyle habits. Participants then received detailed instructions and maintained a three-day food diary to enable approximate evaluation of dietary intake quality and estimated adherence to the Mediterranean diet pattern.

Methods

1. Anthropometric methods
Anthropometric parameters were collected to assess body size, body composition, and the risk of developing non-communicable chronic diseases. Body height was measured using a SECA 217 mobile stadiometer, with participants standing without shoes and the head positioned in the Frankfort horizontal plane. Body mass and body composition were assessed using an OMRON BF511 diagnostic scale, which operates on the BIA principle. Participants stood barefoot on the four foot electrodes, wore light clothing without jewelry, and held the four hand electrodes at chest level. This analyzer provided data for body mass, BMI, muscle mass percentage, fat mass percentage, and visceral fat amount. Circumferences of the neck, upper arm, waist, hips, and lower leg were measured to the nearest 0.1 cm using a non-elastic measuring tape placed at the midpoint of the neck, upper arm, and lower leg, the narrowest point of the waist, and the widest part of the hips. The following ratios were then calculated to evaluate fat mass distribution: WHR, which classifies fat mass distribution as android (accumulating more fat in the abdominal area) or gynoid type (accumulating more fat in the hip area); WHtR, which indicates fat mass distribution; and NHR, which serves as an indicator of metabolic syndrome risk.

2. Dynamometry
Muscle strength was assessed in the dominant hand by measuring handgrip strength with two instruments: the analog *Jamar* and the digital *Camry* EH101 dynamometer. During data collection, participants sat in a chair with a backrest. The knees, hips, and elbow of the dominant hand were each positioned at a 90° angle. The dynamometer handle size was individually adjusted for each student as needed. Measurements with each dynamometer were performed in three consecutive attempts, separated by equal time intervals. The final strength value was calculated as the arithmetic mean of the three recorded measurements.

3. Dietary methods
On the day of anthropometric and muscle strength assessment, participants completed a brief 15-item questionnaire to collect comprehensive information about their dietary and lifestyle habits. The questionnaire included questions on the presence of chronic diseases and current medication use, lifestyle habits (smoking, weekly alcohol consumption, and daily coffee consumption), use of dietary supplements (vitamin/mineral supplements, meal replacements, energy/protein bars, and protein blends), consumption of protein-enriched foods, adherence to special dietary regimens, and the frequency of daily meal consumption (breakfast, lunch, snack, and dinner), rated from “never” to “seven times per week.”
To examine dietary habits and quality in more detail, participants maintained a three-day food diary on three non-consecutive days (two weekdays and one weekend day). To ensure accuracy, participants received detailed instructions. All consumed food and drink quantities were determined either by weighing with a kitchen scale or by estimation using standard kitchen utensils (e.g., spoon, teaspoon, glass, cup). For portioned

packaged products, information was taken directly from the label declarations. For all complex dishes, participants provided detailed recipes, and standard recipes were used when specific preparation instructions were unavailable. Consumed foods and drinks were entered into the *Prehrana* software (Infosistem d.d., Zagreb), which is based on national food composition tables (Kaić-Rak and Antonić, 1991). To assess adherence to the Mediterranean dietary pattern, the MDQI was calculated for each participant. The MDQI includes seven components: fruit and vegetables (g), cereals and products (g), fish (g), meat (g), olive oil (mL), cholesterol (mg), and saturated fatty acids (% kJ). Each component was scored 0, 1, or 2 points based on compliance with dietary guidelines (scoring method detailed in Table 1). The total MDQI score, ranging from 0 to 14 points, was obtained by summing the scores of the seven components (Bach et al., 2006). A lower total score indicates better dietary quality. Nutrition quality was classified into four categories: good nutrition (0–4 points), medium-good nutrition (5–7 points), medium-poor nutrition (8–10 points), and poor nutrition quality (11–14 points).

Table 1. MDQI scoring method (*according to* Bach et al., 2006)

Parameters of MDQI	Number of points		
	0	**1**	**2**
fruits and vegetables (g)	> 700	700 - 400	< 400
cereals and products (g)	> 300	300 - 100	< 100
fish (g)	> 60	60 - 30	< 30
meat (g)	< 25	25 - 125	> 125
olive oil (mL)	> 15	15 - 5	< 5
cholesterol (mg)	< 300	300 - 400	> 400
saturated fatty acids (% kJ)	< 10	10 - 13	> 13

Results and discussion

General participants' information

A total of 38 students (N = 38), aged 20 to 28 years (mean 24.4 ± 1.4 years), participated in this study. The mean age by faculty was 25.1 years for EFZG students, 24.1 years for PFZG and KIF students, and 24 years for PBF students. Regarding health status, only one individual reported an autoimmune disease. Additionally, six participants were using hormonal therapy, and one participant was using antidepressant medication at the time of data collection. The majority of participants, specifically 42%, reported living with their parents, while 13% lived in an apartment with roommates. Students living with their parents were more likely to consume home-prepared food compared to those living alone, with roommates, or in student dormitories. Regular physical activity was defined as engaging in organized training, gym, or home exercise at least once per week. Overall, 63% of participants reported being regularly physically active, while 37% were not regularly active. EFZG students had the lowest rate of regular physical activity, with only 37.5% regularly active. Only 40% of PFZG students reported regular physical activity. In contrast, 70% of PBF students were regularly physically active. As expected, all KIF students reported being regularly physically active. These results are consistent with findings from student populations internationally, where physical activity prevalence commonly ranges from approximately 55% to 65%, (Chaabana et al., 2022; Alhammad et al., 2023), and are also comparable to Croatian data showing that around 70% of university students meet recommended physical activity guidelines (Pedišić et al., 2014).

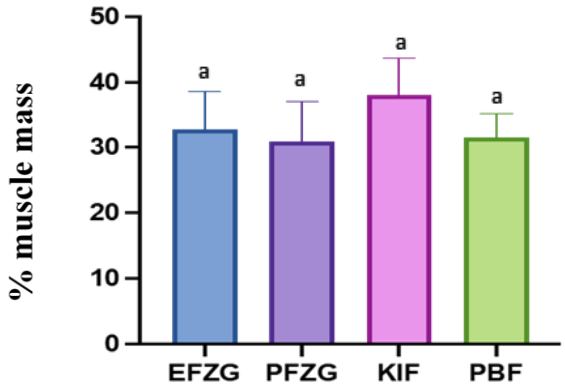
Body composition

The analysis of basic anthropometric parameters (Table 2) revealed that all students' groups were within the normal range for body mass index (BMI).

Table 2. Average anthropometric parameters of the study population

Anthropometric parameter	Faculty name*			
	EFZG	**PFZG**	**KIF**	**PBF**
Average body mass (kg)	72.86	68.82	67.53	63.9
Average body height (cm)	174.1	169.4	172.3	166.9
Average BMI (kg/m^2)	23.75	23.80	22.57	22.67

**EFZG – UNIZG Faculty of Economics; PFZG – UNIZG Faculty of Law; KIF – UNIZG Faculty of Kinesiology; PBF – UNIZG Faculty of Food Technology and Biotechnology*



*EFZG – UNIZG Faculty of Economics; PFZG – UNIZG Faculty of Law; KIF – UNIZG Faculty of Kinesiology; PBF – UNIZG Faculty of Food Technology and Biotechnology

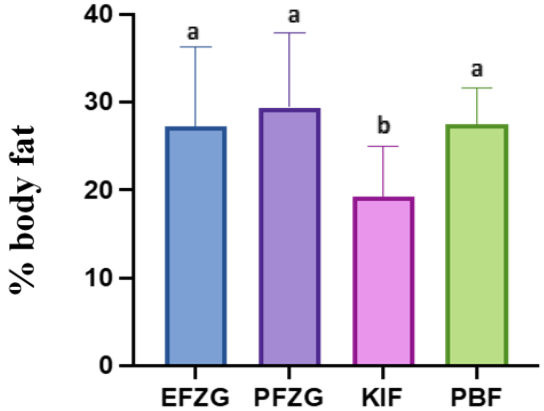
Figure 1. Average proportion of muscle mass (%) in the studied population

EFZG students had the highest average body mass (72.86 kg) and height (174.1 cm), while PBF students reported the lowest values (63.9 kg and 166.9 cm). However, BMI is a primary indicator of body composition. The highest average BMI was observed in PFZG students (23.80 kg/m²), while the lowest was recorded in KIF students, despite the expectation that a group with a majority of male participants would have a higher BMI. Similar BMI results were found in an earlier study of Croatian students, where the average BMI was 23.19 ± 3.59 kg/m² (Štefan et al., 2017). Although the group BMI averages were adequate, 11 of the 38 participants had BMI values above 24.9 kg/m², placing them in the overweight category. A more accurate representation of body composition is provided by the proportions of muscle and fat mass, as well as NHR, WHR, and WHtR ratios.

Fig. 1. shows the results of the average proportion of the muscle mass expressed in percentages (%).

As expected, KIF students had the highest average muscle mass proportion at 38.01 ± 5.31%, classifying this group as having a very high amount of muscle mass. In contrast, PFZG students had the lowest average muscle mass proportion at 30.97 ± 5.79%. PBF and EFZG students had intermediate values of 31.60 ± 3.39% and 32.75 ± 5.38%, respectively. The average muscle mass in previous research conducted by Štefan et al. (2017) was similar to that obtained in this study, at 32.12 ± 6.07%. The lower muscle mass in the PBF student group is likely due to the high proportion of female students, who generally have lower relative muscle mass compared to men.

Analysis of average fat mass proportions using the Kruskal-Wallis test showed a statistically significant difference among the four participant groups (Fig. 2). PFZG students had the highest average fat mass proportion at 29.44 ± 8.06%. Within this group, male participants had an average body fat mass of 22.4%, while females had 32.46%. EFZG students followed with an average of 27.26 ± 8.41%, with males at 25%—classified as having a very high body fat percentage – and females at 28.62%. A similar result was observed for male PBF participants, who also fell into the very high body fat percentage group with an average of 25.95%, while female participants in this group maintained an adequate average body fat percentage (27.62 ± 3.75%). In contrast, KIF students had the lowest average body fat percentage at 19.32 ± 5.42%, which aligns with adequate values for both sexes. Previous research found that the average percentage of body fat in students is around 27.86 ± 7.90% (Štefan et al., 2017), which is higher than the average of the surveyed population.



*EFZG – UNIZG Faculty of Economics; PFZG – UNIZG Faculty of Law; KIF – UNIZG Faculty of Kinesiology; PBF – UNIZG Faculty of Food Technology and Biotechnology

Figure 2. Body fat percentage (%) in the studied population

Visceral fat values between 0 and 9 are classified as normal. The highest average visceral fat level was observed in PFZG students, with a mean of 5.2 ± 3.63; two students exceeded the normal range, increasing their risk of chronic non-communicable diseases. A similar average was found among PFZG students (5.1 ± 3.18), with one participant showing an elevated level. In contrast, the average visceral fat levels for KIF and PBF students were 4.3 ± 1.73 and 4.4 ± 2.37, respectively, with all individuals maintaining values within the normal range. Because measurements relied on the OMRON BF511 body composition analyzer and could not be standardized (ideally taken in the morning, before eating, and after voiding), most measurements were conducted in the afternoon due to students' obligations, which may have introduced variability compared to actual results.

A one-way ANOVA test indicated that there was no statistically significant difference between the four groups of participants for the measured circumference parameters (neck, upper arm, waist, hip, and lower leg), and the results are shown in Table 3.

Table 3. Results of circumference measurements in the studied population

Parameters	Faculty name*			
	EFZG	PFZG	KIF	PBF
Neck circumference (cm)	33.8	33.73	35.29	32.43
Upper arm circumference (cm)	29.39	28.31	29.18	28.22
Waist circumference (cm)	77.36	74.84	74.26	70.02
Hip circumference (cm)	102.30	99.06	96.36	97.01
Lower leg circumference (cm)	37.83	36.61	36.47	35.91

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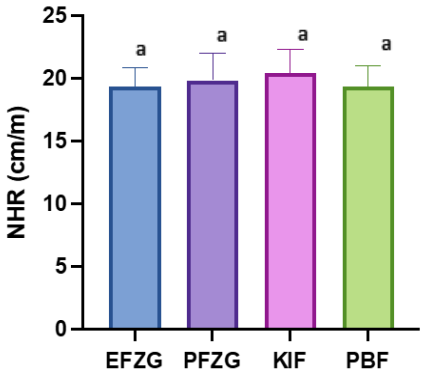
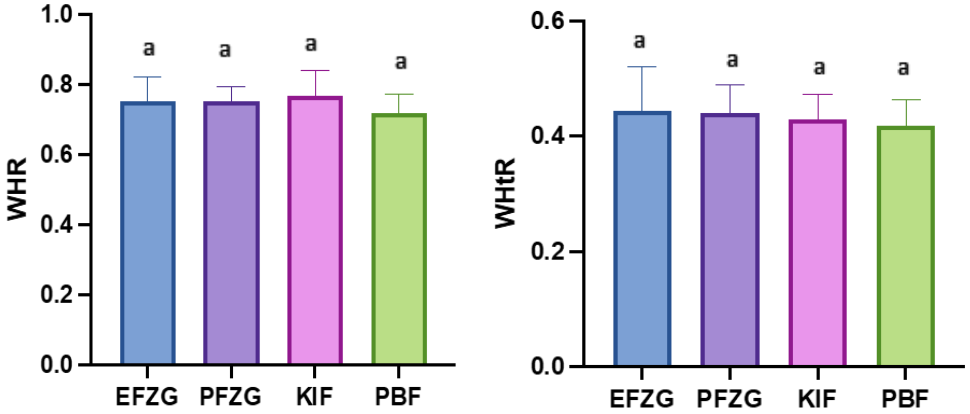


**EFZG – UNIZG Faculty of Economics; PFZG – UNIZG Faculty of Law; KIF – UNIZG Faculty of Kinesiology; PBF – UNIZG Faculty of Food Technology and Biotechnology*

Figure 3. NHR values in the studied population

The NHR (Fig. 3), an indicator of metabolic syndrome risk (Selvan et al., 2016) calculated by relating neck circumference (cm) to body height (m), also showed no statistically significant difference among the four groups according to ANOVA. The KIF students had the highest average NHR value (20.45 ± 1.76 cm/m), likely due to the majority of male participants in this group. The PBF and EFZG students had nearly identical average NHR values (19.37 ± 1.53 cm/m and 19.37 ± 1.34 cm/m), while the PFZG students averaged 19.88 ± 2.05 cm/m. Eleven participants (nine men and two women) had elevated NHR values indicating a significant risk of metabolic syndrome (Selvan et al., 2016). Compared to the World Health Organization guidelines (≤ 0.85 for women and ≤ 0.90 for men), the WHR analysis revealed no statistically significant difference among the four groups. All students were within the established non-risk category (WHO, 2011), with the lowest average WHR observed among PBF students. Similarly, no significant intergroup difference was found for the WHtR. Despite the group averages, four students had WHtR values above 0.5, indicating an elevated risk for chronic non-communicable diseases (Fig. 4).



**EFZG – UNIZG Faculty of Economics; PFZG – UNIZG Faculty of Law; KIF – UNIZG Faculty of Kinesiology; PBF – UNIZG Faculty of Food Technology and Biotechnology*

Figure 4. WHR and WHtR values in the studied population

Muscle strength

Muscle strength was measured on the dominant hand using two devices: the analog Jamar and the digital Camry hand-held dynamometers (Table 4). The average of three consecutive measurements from each device was used for data analysis. The primary goal was to compare the measurements between the two devices and assess their consistency in measuring handgrip strength among young adults.

A one-way ANOVA test revealed a statistically significant difference in handgrip strength among the four groups. As expected, due to a higher physical activity rate, KIF students showed the highest average handgrip strength on both devices, while PFZG students recorded the lowest averages.

To compare the consistency of the *Jamar* and *Camry* dynamometers, participants were divided into two groups based on assumed physical activity levels: Group 1 (higher activity) included KIF and PBF students (Fig. 5), and Group 2 (lower activity) included PFZG and EFZG students (Fig. 6). In Group 2, the average strength was 29.29 ± 9.13 kg (*Jamar*) and 25.50 ± 9.72 kg (*Camry*). In Group 1, the average strength was 36.73 ± 12.53 kg (*Jamar*) and 35.00 ± 11.55 kg (*Camry*). In both groups, the results did not indicate a statistically significant difference between the handgrip strength measurements taken with the *Jamar* and *Camry* dynamometers, indicating that both devices have comparable precision when used to assess handgrip strength in the young adult population. In the study by Lupton-Smith et al. (2022) involving adults, two identical measuring devices were compared, and the results were consistent with those of this study. Additionally, the study found that the digital *Camry* device had good reproducibility compared to the analog *Jamar* (Lupton-Smith et al., 2022).

Table 4. Muscle strength in the studied population

Hand-held dynamometers	Faculty name*			
	EFZG	PFZG	KIF	PBF
***Camry* (kg)**	27,72	30,93	40,68	29,32
***Jamar* (kg)**	26,35	31,63	42,23	31,22

**EFZG – UNIZG Faculty of Economics; PFZG – UNIZG Faculty of Law; KIF – UNIZG Faculty of Kinesiology; PBF – UNIZG Faculty of Food Technology and Biotechnology*

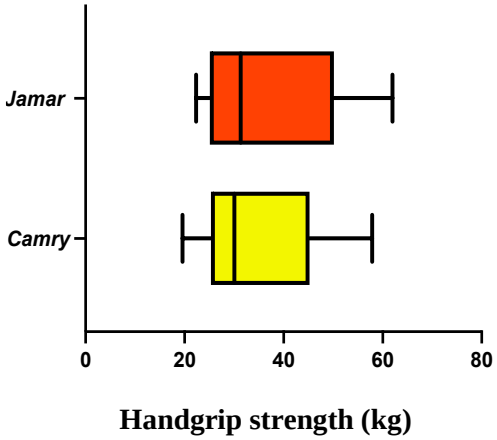


Figure 5. Handgrip strength in Group 1

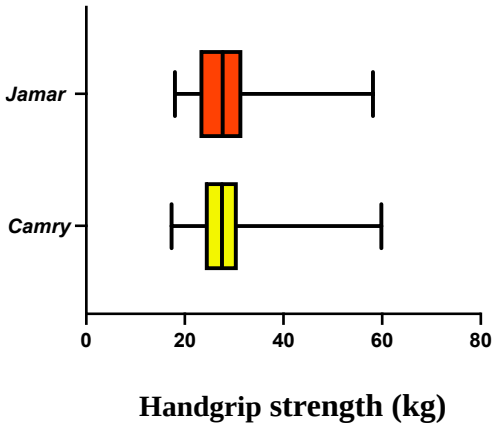


Figure 6. Handgrip strength in Group 2

Dietary quality

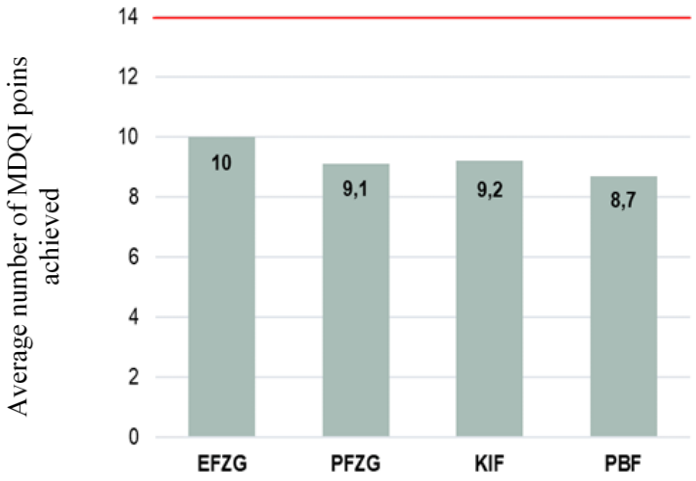
The quality of the participants' diets was evaluated by analyzing three-day food diaries. Using the *Prehrana* software, the average individual intakes of energy, macronutrients, and micronutrients were determined. To provide a more accurate representation of daily consumption, the average daily intake was calculated relative to the participants' average body mass (TM). KIF students recorded the highest energy intake per kilogram of body mass (29.62 kcal/kgTM), followed by PFZG students (28.52 kcal/kgTM), PBF students (25.94 kcal/kgTM), and EFZG students (25.67 kcal/kgTM), who consumed the least energy. he average intake of macronutrients (carbohydrates, fat, and protein) is shown in Table 5. While PBF students had the highest proportion of carbohydrates in total energy intake and KIF students had the lowest, no group met the recommended DRV for total carbohydrates or dietary fiber (25 g/day; EFSA, 2019).

Table 5. Average macronutrients intake in the studied population

Faculty name	Carbohydrates (% total energy intake)	Fat (% total energy intake)	Proteins (g/kgTM)
EFZG	37.95	43.28	1.05
PFZG	37.66	43.32	1.39
KIF	37.03	44.78	1.42
PBF	41.77	39.08	1.24
Average	38.60	42.62	1.28

**EFZG – UNIZG Faculty of Economics; PFZG – UNIZG Faculty of Law; KIF – UNIZG Faculty of Kinesiology; PBF – UNIZG Faculty of Food Technology and Biotechnology*

The compliance of the participants' diets with the Mediterranean dietary pattern was evaluated using the MDQI calculated from the three-day food diaries. An average MDQI score was determined for each student based on the daily values recorded. The MDQI scores, compared across the four groups (Fig. 7), classified nutritional quality into four categories: optimal (0 to 4 points), moderately good (5 to 7 points), moderately poor (8 to 10 points), and undesirable (11 to 14 points). The MDQI scores for all four groups indicated that their dietary compliance fell within the moderately poor category. The results are similar to those of Štefan et al. (2017), where low adherence to the Mediterranean diet was observed among young adults.



**EFZG – UNIZG Faculty of Economics; PFZG – UNIZG Faculty of Law; KIF – UNIZG Faculty of Kinesiology; PBF – UNIZG Faculty of Food Technology and Biotechnology*

Figure 7. MDQI scores compared among the four groups in the studied population

Conclusion

University students' health habits vary significantly by field of study, with those in science, biotechnology, and kinesiology showing more favorable trends in physical activity, body composition, muscle strength, and diet quality than students in social sciences and humanities. Additionally, both *Camry* and *Jamar* dynamometers provide equally valid results for assessing handgrip strength in young adults.

Limitations of this study include the relatively small sample size and self-reported dietary records, which may introduce reporting bias. Future studies should use larger cohorts and objective biomarkers of nutrient intake.

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INFLUENCE OF HIGH-VOLTAGE ELECTRICAL DISCHARGE ON THE OIL BINDING CAPACITY OF SPELT CHAFF AND WHEY

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Summary

Oil binding capacity (OBC) is a key property in the formulation of oleogels (structured vegetable oils without trans or saturated fatty acids). Food industry by-products are promising structuring agents for oleogels, as they can bind oil. With appropriate treatment, OBC can be improved. The green technology known as High-Voltage Electrical Discharge (HVED) has shown potential for modifying the structure of materials for this purpose. In this study, spelt chaff and whey were treated with HVED under different conditions (time and frequency). HVED successfully enhanced OBC values for lignocellulosic material spelt chaff, while it did not have a significant effect on protein-rich material whey. The influence of the process parameters was determined by response surface methodology (RSM). The results showed that the highest OBC value for spelt chaff was achieved when treated for 15 minutes at 40 Hz. These findings suggest that HVED can be used as a green physical treatment for material modification, supporting the circular economy, sustainability, and the valorisation of by-products.

Keywords: oil binding capacity, high voltage electrical discharge, spelt chaff, whey, response surface methodology

Introduction

Nowadays, structuring edible oil by physical sorption onto materials has attracted interest in the scientific community. The resulting structured oil systems (oleogels) enable the development of novel food products with improved nutritional value, being lower in trans and saturated fatty acids. Therefore, the ability of materials to bind oil is a crucial property in formulating such oleogels. Oil sorption (absorption and adsorption), also known as oil binding capacity, is defined as the enrichment of a material with oil in the interfacial layer, followed by the penetration of oil into the solid phase (Rouquerol et al., 2012; Patel and Dewettinck, 2016). Consequently, food researchers are seeking suitable materials for this purpose. Wahid et al. (2026) proposed using underutilised by-products to develop cost-effective materials, thereby valorising by-products at the same time. In this way, the concept of a circular economy can be established through reduction, reuse, and recycling in the food supply chain (de Oliveira et al., 2021).

If appropriate physical treatment is applied, materials can be modified into more effective structuring agents for oleogel production by increasing oil binding capacity during treatment. Yin et al. (2021) reported that physical treatments such as ball milling or high-pressure homogenisation can create a loose, porous network structure that enhances oil binding capacity. They highlighted the importance of particle size and the microstructure of insoluble dietary fibre in relation to oil binding capacity. According to Krause et al. (2023), protein powders with small particle size and low density tend to adsorb more oil than those with high density.

Since High-Voltage Electrical Discharge (HVED) demonstrates potential for material modification, as reported by Ačkar et al. (2022), where starch was modified, it suggests that this physical treatment can increase oil binding capacity. HVED is considered an innovative green technology, which shows potential to extract various bioactive compounds from biological materials, including by-products from the food industry. This method offers several advantages over conventional extraction techniques, such as cost-effectiveness, reduced extraction times, and low operating temperatures. During HVED treatment, high energy is generated in the aqueous suspension positioned between a grounded electrode and a high-voltage needle electrode. An electron avalanche and several secondary phenomena – such as shock waves, cavitation, and liquid turbulence – are initiated by a plasma channel formed by a rapid electrical discharge, resulting in mechanical cell disintegration and electroporation. These secondary phenomena accelerate the extraction of biomolecules (Pastor et al., 2025; Boussetta et al., 2013). In addition to its extraction and modification potential, HVED may also offer the possibility of food decontamination (Kiš et al., 2020).

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Spelt (*Triticum spelta*) is one of the oldest known grains. In the past, spelt's popularity declined due to lower crop yields and the expensive dehulling process, compared to high-yielding common wheat (*Triticum aestivum*), where the hulls are separated from the kernel immediately at harvest. However, spelt is more resistant to drought and fungal pathogens than bread wheat, which is why it is becoming more significant in agriculture, as its resistance is suitable for organic farming systems. As environmental awareness has increased, spelt is receiving renewed attention because it requires less pesticide and fertiliser for cultivation. Additionally, spelt grains are superior in protein content, soluble dietary fibre, fat, and minerals. However, spelt has lower gluten quality, which is important for dough production. If the soil is rich in nutrients, spelt will produce lower yields compared to common wheat. After the dehulling process, spelt chaff is obtained as a by-product. As this material can be high in various mycotoxins, it is not recommended for use as feed or food without prior treatment. According to Wiwart et al. (2017), spelt chaff can be utilised in energy production due to its high calorific value (Kiš et al., 2017; Wiwart et al., 2017; Wanic et al., 2024).

Whey is a liquid by-product obtained during cheese production. In the production of one kilogram of cheese, approximately nine litres of whey remain as residue. Although whey has high nutritional value, particularly because of its proteins, significant investment is required for its valorisation. Large quantities in the ecosystem can negatively affect the environment if disposed of as waste, since the biological oxygen demand of whey is very high (40,000 mg/kg). Depending on the method of cheese production, whey can be either sweet if enzymes are used, or acidic if acid is used for coagulation. Besides water, which makes up 93% of whey, the main component is lactose, accounting for 70–72% of the dry matter. Whey proteins are present at 8–10%, while minerals make up 12–15% of the dry matter. With appropriate technology, whey can be converted into valuable products such as whey cheeses (e.g. Ricotta), beverages, whey protein isolate, etc. (Jelen, 2011; Slačanac and Lučan, 2017).The aim of this study was to determine the potential of high voltage electrical discharge as a physical treatment for modifying biowaste (spelt chaff and whey) to increase oil binding capacity. The goal is to achieve the highest possible outcome by optimising process parameters (HVED treatment time and frequency) with response surface methodology.

Materials and methodology

High voltage electrical discharge

Spelt chaff was obtained from EKO-JAZO (Croatia), while whey, dried into powder, was provided by Belje Plus (Croatia). Spelt chaff was ground into powder using an ultra centrifugal mill (ZM 200, Retsch, Germany). The prepared materials were modified using high voltage electrical discharge (HVED). Raw materials were suspended in ultrapure water. Based on preliminary research, 60 g of whey was suspended in 240 mL of water, and 20 g of spelt chaff in 280 mL of water. A grounded electrode and a high-voltage needle (point-to-plane geometry) were positioned 0.5 cm apart. The gas flow (synthetic air) was set at 2 m/s. After treatment, the suspension was centrifuged for 20 min at 3000 RPM, the supernatant was decanted, and the treated samples were oven-dried at 50 °C. Different frequencies and treatment times were tested.

Oil binding capacity

Oil binding capacity (OBC) was determined using AACC Method 88–04 (AACC, 1983). Material (2.5 g) is suspended in sunflower oil (30 mL). After 30 minutes, the resulting suspension was centrifuged at 3000 rpm for 15 minutes at ambient temperature. Excess oil was decanted, and the precipitate was weighed together with the Falcon tube. The value for oil binding capacity was calculated using Eq. (1), defined as the ratio of the precipitate mass after centrifugation to the total mass of the sample, indicating how much oil was bound to the sample.

$$OBC = \frac{(weight\ of\ precipitate\ after\ centrifugation)}{(weight\ of\ the\ sample)} \quad (1)$$

Statistical Analysis

To clarify the influence of the two variables (frequency and treatment time) on oil binding capacity, a second-order polynomial regression model is used, as shown in Eq. (2), in which:

Y – anticipated outcome of oil binding capacity; β_0 – intercept; β_1, β_2 – linear coefficients; β_{11}, β_{22} – quadratic coefficients; β_{12} – interaction coefficient; A, B – variables (HVED treatment time and frequency, respectively); ε – random error term.

$$Y = \beta_0 + \beta_1 A + \beta_2 B + \beta_{11} A^2 + \beta_{22} B^2 + \beta_{12} AB + \varepsilon \quad (2)$$

Data analysis was conducted using R software (R Core Team, 2022). The significance level was set at a probability value of 0.05. The F-test, Shapiro-Wilk test, and ncvTest (non-constant variance score test) from the package „car“ (Fox and Weisberg, 2019) were used to assess the validity of the regression model. The ncvTest was used to detect heteroscedasticity in the regression model, while the Shapiro-Wilk test was applied to the studentized residuals to check for normality. The t-test was used to assess the significance of the regression coefficients. The F-test of overall significance was conducted to determine whether the regression model provided a better fit than a model with no independent variables. In this study, response surface methodology (RSM) was applied to identify the region in which the maximum value of the response function (oil binding capacity) is located. By employing an appropriate experimental design and an approximation model, the effects of two factors (HVED treatment time and frequency) and their interactions on the response were systematically explored. Based on the fitted model, the optimal region was determined in order to provide a foundation for more refined experimental design and subsequent investigation.

Results and discussion

Table 1. Experimental design and response values for oil binding capacity of spelt chaff (g/g)

Run	Factor 1 A: HVED treatment time (min)	Factor 2 B: Frequency (Hz)	Response: Oil binding capacity (g/g)	Run	Factor 1 A: HVED treatment time (min)	Factor 2 B: Frequency (Hz)	Response: Oil binding capacity (g/g)
1	15	60	5.60	29	15	40	7.86
2	15	60	5.43	30	15	40	7.58
3	15	60	6.78	31	15	40	7.98
4	15	60	6.89	32	15	40	7.20
5	15	40	7.91	33	10	50	8.68
6	15	40	7.76	34	10	50	8.73
7	15	40	8.39	35	10	50	8.71
8	15	40	7.87	36	10	50	8.32
9	25	40	6.19	37	15	40	8.80
10	25	40	6.77	38	15	40	8.57
11	25	40	7.16	39	15	40	8.64
12	25	40	6.98	40	15	40	8.14
13	10	30	7.90	41	15	40	8.20
14	10	30	8.02	42	15	40	8.24
15	10	30	7.73	43	15	40	8.55
16	10	30	7.90	44	15	40	8.57
17	20	30	7.98	45	15	40	8.69
18	20	30	8.12	46	15	40	8.12
19	20	30	8.54	47	15	40	7.96
20	20	30	8.48	48	15	40	8.20
21	15	20	7.64	49	20	50	8.53
22	15	20	7.59	50	20	50	8.06
23	15	20	6.61	51	20	50	7.98
24	15	20	6.72	52	20	50	7.96
25	5	40	5.97	53	15	40	7.54
26	5	40	5.67	54	15	40	7.44
27	5	40	7.42	55	15	40	7.24
28	5	40	7.13	56	15	40	7.01

Oil binding capacities for untreated samples were 5.74 ± 0.001 g/g for spelt chaff and 2.01 ± 0.01 g/g for whey. According to the results shown in Tables 1 and 2, whey had noticeably lower OBC values than spelt chaff after HVED treatment, indicating that HVED is less effective for protein-rich materials than for lignocellulosic materials. According to Sangnark and Noomhorm (2003), the oil binding capacity of

sugarcane bagasse increased after alkaline hydrogen peroxide treatment compared with the raw material, as a result of lignin destruction in the lignocellulosic material. Since Miranda et al. (2020) reported that plasma-in-water treatment caused removal of lignin from biomass, it suggests that HVED treatment caused partial removal of lignin in spelt chaff, thus increasing oil binding capacity.

Table 2. Experimental design and response values for oil binding capacity of whey (g/g)

Run	Factor 1 A: HVED treatment time (min)	Factor 2 B: Frequency (Hz)	Response: Oil binding capacity (g/g)	Run	Factor 1 A: HVED treatment time (min)	Factor 2 B: Frequency (Hz)	Response: Oil binding capacity (g/g)
1	14	30	2.27	25	14	50	2.39
2	14	30	2.36	26	14	50	2.39
3	14	30	2.33	27	14	50	2.38
4	14	30	2.29	28	14	50	2.31
5	16	60	2.38	29	14	50	2.47
6	16	60	2.51	30	14	50	2.39
7	16	60	2.28	31	14	50	2.27
8	16	60	2.28	32	14	50	2.30
9	14	50	2.55	33	12	60	2.72
10	14	50	2.72	34	12	60	2.68
11	14	50	2.26	35	12	60	2.24
12	14	50	2.34	36	12	60	2.27
13	14	50	2.68	37	10	50	2.20
14	14	50	2.84	38	10	50	2.28
15	14	50	2.84	39	10	50	2.24
16	14	50	2.69	40	10	50	2.18
17	14	50	3.20	41	16	40	2.61
18	14	50	3.08	42	16	40	2.61
19	14	50	2.84	43	16	40	2.31
20	14	50	2.71	44	16	40	2.32
21	14	50	2.41	45	12	40	2.32
22	14	50	2.46	46	12	40	2.39
23	14	50	2.45	47	12	40	2.84
24	14	50	2.37	48	12	40	2.87

Statistical Analysis

In the case of spelt chaff, the Shapiro-Wilk test ($W = 0.97227$, $p = 0.2228$) failed to reject the null hypothesis ($p > 0.05$), confirming that studentized residuals follow a normal distribution. The ncvTest confirmed homogeneity of variance for spelt chaff ($\chi^2 = 2.3624$, $Df = 1$, $p = 0.1243$). The residual analysis confirmed model adequacy, as both homoscedasticity and normality were achieved. The regression model for spelt chaff was statistically significant ($F(5, 50) = 7.473$, $p = 2.664 \times 10^{-5}$; $p < 0.001$). The linear and quadratic regression coefficients were also statistically significant ($p < 0.05$), while the interaction coefficient was not (Table 3). This indicates that the predictors had a significant effect on the response (OBC), but their effects were independent. Although the regression model assumptions were met, the regression model developed for spelt chaff explained only approximately 40% of the variability, as indicated by the low adjusted coefficient of determination ($R^2 = 0.3705$). This result suggests that predictors (HVED time treatment and frequency) were not the only parameters affecting changes in OBC. Variability in the OBC occurred by other factors beyond this study. This may be explained by the heterogeneous fibre sample whose particle size varied. Wang et al. (2020) stated that conventional methods for oil absorption capacity can encounter issues due to material loss during the decanting process and reabsorption of oil after centrifugation. According to Locke et al. (2006), point-to-plane geometry can be problematic since the volume of the plasma discharge active region is not uniform throughout the suspension, suggesting that not every particle is treated equally. All of these factors could explain why most of the variability was unexplained by the predictors.

The second-order polynomial equation for the oil binding capacity of spelt chaff is given in Eq. 3. Analysis of the fitted model indicated that the stationary point is a maximum. The optimum condition was achieved at 15.63 min (HVED treatment time) and 37.77 Hz (frequency). The corresponding predicted maximum value for oil

binding capacity was 8.243 g/g, as shown in Fig 1. and Fig 2. Compared to untreated spelt chaff, the oil binding capacity increased by 43.6%, indicating successful modification of spelt chaff.

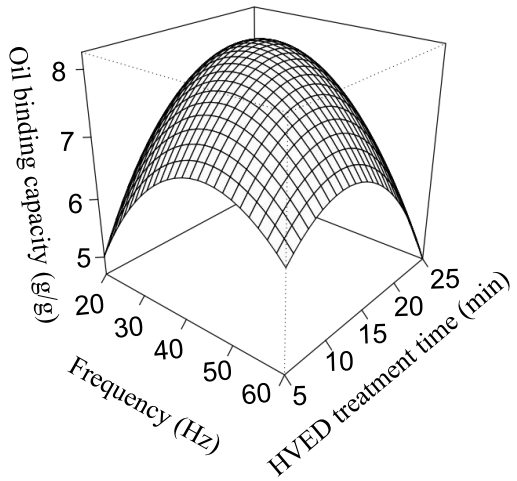

Figure 1. Response surface plot showing the effect of HVED treatment time and frequency on oil binding capacity of spelt chaff

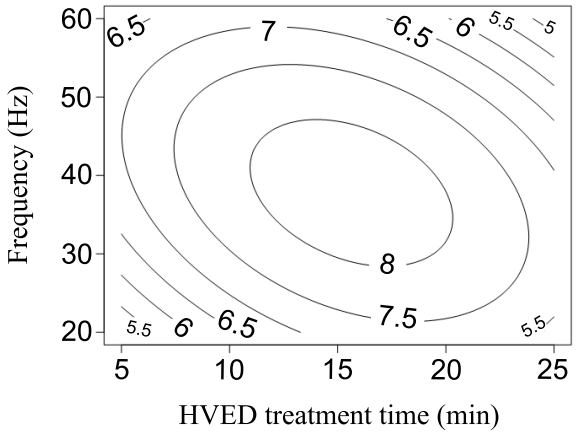

Figure 2. Contour plot showing the effect of HVED treatment time and frequency on oil binding capacity of spelt chaff

Table 3. Estimated regression coefficients with standard errors for oil binding capacity of spelt chaff

Coefficients	Estimate	Std. Error	t-value	p-value
(Intercept)	-1.8541813	2.429989	-0.763	0.449024
HVED treatment time (A)	0.5548533	0.157771	3.517	0.000939
Frequency (B)	0.3049932	0.074578	4.090	0.000157
A^2	-0.0125203	0.002719	-4.606	0.0000286
B^2	-0.0031421	0.000680	-4.623	0.0000269
A×B	-0.0043262	0.003342	-1.295	0.201413

$$Y = -1.8542 + 0.5549\,A + 0.3050\,B - 0.0125\,A^2 - 0.0031\,B^2 - 0.0043\,AB + \varepsilon \quad (3)$$

The F-test for the whey regression model ($F(5, 42) = 1.888$, $p = 0.1169$; $p > 0.05$) showed that the model with no independent variables fits the data as well as the regression model. In addition, the coefficients in the regression models for whey were not statistically significant ($p > 0.05$). Overall, HVED treatment in the case of protein-rich material whey did not show a statistically significant effect on oil binding capacity. Consequently, reliable response surface analysis could not be established in this case.

Conclusion

Under the conditions applied in this research, high-voltage electrical discharge (HVED) successfully increased oil binding capacity of spelt chaff. Using response surface methodology, the highest OBC value was observed at 15 min and 40 Hz, which was 43.6% higher compared to the untreated sample. In contrast, the oil binding capacity of whey was not significantly altered by HVED treatment, which may be due to structural differences between lignocellulosic and protein-rich materials. Overall, HVED, as a green physical treatment, is a promising non-thermal technology for enhancing the properties of selected food industry by-products, especially lignocellulosic materials, into potential structuring agents for oleogel production. Additional research is needed to determine if the lignin loss indeed occurred due to the application of HVED. It is also necessary to identify additional factors that influence OBC besides HVED treatment time and frequency, such as different HVED device geometries and the effect of particle size heterogeneity.

Acknowledgement

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PHYSICAL ACTIVITY, SEDENTARY BEHAVIOUR, AND SELECTED DIETARY HABITS OF MIDDLE SCHOOL–AGED CHILDREN IN THE UNA-SANA CANTON (BOSNIA AND HERZEGOVINA)

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Summary

The aim of this study was to analyse physical activity, sedentary behaviour, and dietary habits related to meal characteristics among younger school-age children in the Una-Sana Canton (USC), as well as parents' perceptions of their children's habits. The study was conducted on a sample of 300 children (7–11 years old) from seven schools in rural and urban areas, together with their 278 parents. Data were collected using age-appropriate questionnaires based on 24-hour dietary recall and a Food Frequency Questionnaire (FFQ), while descriptive statistics, ANOVA, and Duncan's post-hoc test were used for data analysis. The results show that 61.7% of children exercise more than twice a week for at least 1 hour per day, while 28.3% exercise less than twice a week or do not exercise at all. Regarding sedentary behaviour, 29.3% of children spend more than two hours per day in front of screens. School meals are not organised in most schools; therefore, an average of 42.9% of children consume a home-prepared meal, while the remaining children consume bakery sandwiches or pastries, snacks from the school kiosk, or commercially available sweet and salty products, all of which represent unhealthy dietary habits. The proportion of children who do not consume anything at school is 2.7%. A total of 49.7% of children eat meals with their families, while parents report that 61.9% of children have one cooked meal and 34.9% have two cooked meals per day. The findings also indicate that 12.6% of parents believe their child is overweight, whereas the actual proportion of children who are overweight or obese is 30.1%, pointing to misperceptions regarding nutritional status in 17.5% of parents. Most parents recognise the importance of healthy eating (93.2%) and are willing to change their habits for their child's health (92.4%); however, 48.9% express the need for additional education.

Keywords: younger school-age children, physical activity, sedentary behaviour, dietary habits

Introduction

The World Health Organization (WHO) published the Guidelines on Physical Activity and Sedentary Behaviour (WHO, 2020), providing a scientifically based framework for promoting physical activity and reducing sedentary lifestyles. It states that physical inactivity is one of the most significant risk factors for the development of chronic non-communicable diseases (NCDs), such as obesity, cardiovascular diseases, type 2 diabetes, and depression, while Guthold et al. (2018) also note an increased risk of certain cancers. Considering WHO data showing that NCDs account for approximately 74% of all deaths worldwide, this issue is regarded as a major public health concern (WHO, 2025), particularly affecting low- and middle-income countries, including Bosnia and Herzegovina (BiH), classified as an upper-middle-income country (WB, 2025). Sedentary behaviour is another key risk factor for NCDs. In recent years, people have been spending increasing amounts of time sitting in front of various screens, which negatively affects health even when individuals engage in physical activity (Owen et al., 2010; Tremblay et al., 2017). In addition, dietary habits are inseparable from health risks, as they can contribute to the development of physical (Craigie et al., 2011; GBD 2019 Risk Factors Collaborators, 2020) and mental disorders that affect quality of life (Opie et al., 2015). All these risks are exceptionally important during childhood and adolescence, as behavioural patterns formed during this period often persist throughout life (Janssen and LeBlanc, 2010).

These issues appear to have become more pronounced in recent years in BiH. A review of the literature reveals studies on the prevalence of obesity in different age groups (Pavlović et al., 2024), showing worrying trends. According to WHO and UNICEF BiH, obesity is present in 8.0% of children aged 5–9 years and 4.6% of adolescents and youth aged 10–19 years. Projections suggest an increase in obesity by 2030 to 29% among children aged 5–9 years and 38.7% among those aged 10–19 years, in the absence of interventions and treatment (WOF, 2018). However, attempts to identify literature addressing physical inactivity, sedentary behaviour, and

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unhealthy dietary habits among younger school-age children in BiH reveal a lack of adequate sources. Various regional or local studies were found, both older and more recent, but they are generally fragmented, methodologically heterogeneous, and notably lacking in systematic, nationally representative research. The first attempt at systematic documentation of dietary patterns was a pilot study within the B&H MENU project, which showed that 66% of adolescents in BiH spend 3 to 8 hours per day sitting, while 73% engage in moderate daily physical activity (Brenjo et al., 2018). Additional challenges relate to institutional and administrative factors in BiH, as the education system is decentralised, limiting the implementation of standardised school-based monitoring programmes. Moreover, almost no schools have a canteen providing warm meals, leaving children dependent on purchasing food from bakeries and other food outlets, which contributes to unhealthy dietary habits.

Taking all these factors into account, there is a need to define the physical activity, sedentary behaviour, and selected dietary habits of younger school-age children in the Una-Sana Canton (USC), and, based on the results, to propose guidelines to the competent Ministry of Education for educational interventions and programmes aimed at encouraging regular physical activity, reducing sedentary time, and improving dietary habits among children and their parents.

Materials and methodology

Participants

This study included 300 younger school-age children from three cities in the Una-Sana Canton (USC), attending seven schools—three in rural areas (49.6%) and four in urban areas (50.4%). The children were aged 7 to 11 years and attended grades two through five. Additionally, 278 parents participated, with 50.7% from rural and 49.3% from urban areas. Statistically significant differences ($p \leq 0.05$) in participant distribution by place of residence were observed in both groups, children and parents. Among the children, 8.7% were classified as underweight, 60.7% as normal weight, 17.0% as overweight, and 13.7% as obese. Statistically significant differences in nutritional status were observed by sex ($p \leq 0.05$), but not by place of residence. Comparisons with USDA's MyPlate recommendations (USDA, 2019) indicated that children exhibited some positive dietary habits (81.3% eat breakfast daily, 69.8% consume three main meals, 52.9% consume two or more servings of fruit, 83.7% drink water as the main beverage), but also some negative habits (52.9% consume only one serving of vegetables, 51.0% less than 250 mL of milk, 69.3% consume insufficient portions of meat, and 46.7% eat sweets and snacks daily) (Osmančević-Muminović, 2024).

Methods

The study was conducted using an age-appropriate questionnaire for children, adapted from similar studies (Lynch et al., 2014; Bilić-Kirin, 2017). The questionnaire was based on 24-hour dietary recall and the Food Frequency Questionnaire (FFQ). In addition to dietary habits, which included characteristics of daily meals, physical activity habits and the number of sedentary hours were also examined. A separate questionnaire was administered to parents to obtain their perceptions of their children's habits. The study was conducted in classrooms during the 2023/2024 school year (September and October) and was anonymous. Data analysis employed descriptive statistics, while statistically significant differences between rural and urban areas were assessed at a significance level of $p \leq 0.05$ using ANOVA to confirm or reject differences, and Duncan's post-hoc test to compare significant differences between responses and variables. Analyses were performed using Microsoft Office 2016 and the demo version of the XLSTAT-Pro 2024 statistical package for MS Office.

Results and discussion

Table 1 presents the survey results (%; ANOVA) for students (questions 1–8) and parents (questions 3–8), noting that question 6 asked parents about preparing school meals, question 7 about the number of cooked family meals, and question 8 was directed only to parents. According to WHO recommendations (2020), children and adolescents should engage in at least 1 hour of physical activity daily. In this study, the question asked how many times per week children exercise for at least 1 hour per day, and on average 61.7% reported exercising more than twice a week. Considering that a similar proportion of children participate in sports training, the typical number of club training sessions (3–4 per week), and the number of hours of physical education at school (2 per week), it can be concluded that approximately 61% of children achieve satisfactory physical activity levels. However, a substantial proportion exercise less than twice a week or not at all (28.3%). Comparing these results with other countries, for example Croatia, where 11- to 15-year-old children and adolescents engaging in at least 1 hour of daily physical activity

range from 13.0% to 30.9% (Pedišić et al., 2022), Montenegro, where 70.96% of children aged 6–9 years have at least 1 hour of active play per day (Masanović et al., 2024), or the COSI study (WHO/COSI, 2020), which reports data for 25 European countries showing that 61.7% to 98.3% of children aged 6–9 years engage in more than 1 hour of active play daily, our findings fall somewhere in between. Literature does not provide a clear EU average for weekly hours of sports activity for children aged 6–10 years; like most studied parameters, available data are fragmented, highlighting the need for coordinated monitoring.

Table 1. Physical activity, sedentary behaviour, and selected dietary habits of children according to children's and parents' responses

Questions	Answers	Parents (%)			Children (%)		
		Rural	Urban	Total	Rural	Urban	Total
(1) Do you practice any sport?	Yes	53.8 ^{Aa}	68.8 ^{Aa}	61.7	-	-	-
	No	46.2 ^{Ba}	31.2 ^{Ba}	38.3	-	-	-
(2) How many times per week are you physically active for at least 1 hour?	Not at all	4.2 ^{Da}	3.8 ^{Ca}	4.0	-	-	-
	Once	24.5 ^{Ba}	14.0 ^{Bb}	19.0	-	-	-
	Two times	16.8 ^{Ca}	14.0 ^{Ba}	15.3	-	-	-
	More than two times	54.5 ^{Aa}	68.2 ^{Ab}	61.7	-	-	-
(3) How many hours per day do you spend in front of various screens?	Not at all, I'm not interested	14.0 ^{Ca}	13.4 ^{Ca}	13.4	17.5 ^{Ca}	16.1 ^{Ca}	16.9
	Every day, 1–2 hours	53.1 ^{Ac}	57.3 ^{Ac}	57.3	60.3 ^{Aa}	61.5 ^{Aa}	60.8
	Every day, more than 2 hours	32.9 ^{Ba}	29.3 ^{Ba}	29.3	22.2 ^{Ba}	22.4 ^{Ba}	22.3
(4) Do you eat breakfast every day?	Yes	75.5 ^{Aa}	86.6 ^{Aa}	81.3	69.5 ^{Ca}	80.3 ^{Cb}	74.8
	No	4.9 ^{Ca}	3.2 ^{Ca}	4.0	0.7 ^{Ba}	2.9 ^{Bb}	1.8
	Sometimes	19.6 ^{Ba}	10.2 ^{Ba}	14.7	29.8 ^{Aa}	16.8 ^{Ab}	23.4
(5) Do you eat at the school canteen?	Yes	38.5 ^{Ba}	49.0 ^{Ba}	44.0	73.0 ^{Aa}	62.0 ^{Aa}	67.6
	No	61.5 ^{Aa}	51.0 ^{Aa}	56.0	27.0 ^{Ba}	38.0 ^{Bb}	32.4
(6) What do you eat at school?	Home-prepared meal	55.9 ^{Aa}	29.9 ^{Bb}	42.3	73.0 ^{Aa}	62.0 ^{Aa}	67.6
	School snack	2.8 ^{Fa}	31.8 ^{Ab}	18.0	27.0 ^{Ba}	38.0 ^{Ba}	32.4
	Sandwich/pastry from a bakery	21.7 ^{Ba}	17.8 ^{Cb}	19.7	-	-	-
	Chips, juice, cookies	4.9 ^{Da}	1.9 ^{Eb}	3.3	-	-	-
	A bit of everything	11.2 ^{Ca}	16.6 ^{Db}	14.0	-	-	-
	None of the above	3.5 ^{Ea}	2.0 ^{Eb}	2.7	-	-	-
(7) How many meals do you eat together with your family members?	Three main meals	42.7 ^{Aa}	56.1 ^{Aa}	49.7	-	-	-
	One meal	12.6 ^{Ca}	8.9 ^{Ca}	10.7	61.0 ^{Aa}	62.8 ^{Aa}	61.9
	Two meals	38.5 ^{Ba}	26.1 ^{Ba}	32.0	36.9 ^{Ba}	32.8 ^{Bb}	34.9
	I don't have time for any	4.2 ^{Da}	5.7 ^{Da}	5.0	2.1 ^{Ca}	4.4 ^{Cb}	3.2
(8) When is your last meal of the day?	Before 6 p.m.	-	-	-	33.3 ^{Ba}	28.5 ^{Bb}	30.9
	Before 8 p.m.	-	-	-	58.9 ^{Aa}	54.0 ^{Aa}	56.5
	Before 10.m.	-	-	-	7.8 ^{Cc}	17.5 ^{Cc}	12.6

Legend: $^{a, b, c, d}$ Different letters within rows and columns indicate significant differences ($p \leq 0.05$) based on Duncan's test.

A similar situation is observed with sedentary behaviour. Although WHO recommendations (2020) do not specify a strict maximum number of hours spent in front of screens, they advise limiting time spent in sedentary activities. Literature shows that screen time varies across countries and age groups. For example, in Croatia, 52% of primary school children spend more than two hours per day in front of screens (Pedišić et al., 2022). The IDEFICS study conducted in eight EU countries reported that children aged 2 to 10 years spend more than 2 hours per day on screens, with 20% on weekdays and 52% on weekends (Santaliestra-Pasías et al., 2014). In contrast, in Montenegro, 72.1% of children aged 6 to 9 years do not exceed two hours of screen time per day (Masanović et al., 2024). Additionally, the COSI study (WHO/COSI, 2020) showed that, on average, 62.0% of children spend less than 2 hours per day in front of screens. According to our research, 29.3% of children spend more than 2 hours per day in front of screens, which again places our results between those of other countries. It should be noted that for question 2, statistically significant differences were observed between rural and urban areas for all response categories.

Table 1 also presents questions and responses (from question 4 onwards) related to dietary habits,

including meals at home and at school. Breakfast is particularly important, as skipping breakfast is among the dietary habits associated with excess weight in children (Wang et al., 2023). For example, Hasanović et al., (2025) investigated the association between breakfast consumption and weight status in children aged 7 to 10 years, concluding that eating breakfast has a protective effect against obesity. In the present study, breakfast skipping was reported by 24.5% of children in rural areas and 13.4% in urban areas, based on children's responses, with no statistically significant differences between areas. According to parents' responses, 30.5% of children in rural areas and 19.7% in urban areas skip breakfast, and these differences were statistically significant. Comparative studies show that 13.1% of children in Germany skip breakfast (Kesztyüs et al., 2017), while in Slovenia 44% and in Spain 8% of children skip breakfast on weekdays, 21% in Greece and 7% in Norway on weekends (Manios et al., 2015). These comparisons suggest that a higher proportion of children in the USC skip breakfast than indicated by these international figures.

School meals are an important part of students' diets in general, including younger school-age children. They can provide up to 60% of daily energy intake and improve overall diet quality, provided they are varied and nutritionally adequate (WHO, 2006). However, research using various food diversity indices shows that the quality of school meals is often insufficient (Ilić et al., 2022; Dello Russo et al., 2023). Unfortunately, in BiH, most schools do not offer organised school meals with a hot option, so children generally rely on meals brought from home, purchases from bakeries, or snacks from small shops offering sandwiches, bakery products, and different sweet and savory food products. Accordingly, our results show that 55.9% of children in rural areas and 29.9% in urban areas consume a home-prepared meal at school. It is interesting to note parents' responses to this question: 73.0% of rural and 62.0% of urban parents reported preparing a school meal for their child, with statistically significant differences observed between areas. On average, 19.7% of children consume a sandwich or pastry from a bakery, 18.0% a snack purchased at the school kiosk, 14.0% a bit of everything, and approximately 2.7% do not consume anything at school.

The last two questions addressed children's meal habits at home, with their families. Children were asked about the number of meals they consume with their family, while parents were asked about the number of cooked meals and the timing of the last meal of the day. Family meals, regardless of meal composition or frequency, are important for the health and psychosocial outcomes of children and adolescents. A higher frequency of shared meals has been associated with protection against poor dietary quality, obesity, risky behaviours, mental health issues, and better academic performance (Snuggs and Harvey, 2023). Although some studies report contradictory findings, for example, Royo et al. (2024) linked a reduced risk of childhood overweight to consuming at least 50% of daily meals with the family. Results from this study show that a large proportion of children (49.7%) eat three main meals with their family. According to parents, 61.9% reported one cooked meal and 34.9% reported two cooked meals. These are satisfactory results, as only 5% of children reported having no family meals. The last meal should, according to recommendations, be 2 to 3 hours before bedtime (NSF, 2025).

For younger school-age children, it is extremely important that parents are well-educated about proper nutrition, as parents essentially define the child's dietary environment and shape eating behaviours that children often carry into adulthood (Savage et al., 2007). For example, the availability of food at home depends on parental food choices when shopping, effectively modelling children's diets, so parental intervention involves reducing the availability of foods that are unhealthy for the child. In addition, setting rules and educating the child is recommended as a strategy for promoting healthy eating habits (Yee et al., 2017). More frequent family meals and well-established mealtime rules (Lehto et al., 2012) have been associated with lower levels of overweight in children.

However, to successfully develop healthy eating habits, parents must be aware of their importance and well-informed about proper child nutrition. Higher parental education has been associated in numerous studies with more favourable dietary habits in children, including higher breakfast consumption, greater intake of water, fruits, vegetables, and dairy products, and lower consumption of fried potatoes, sugary drinks, processed foods, and snacks (Fernández-Alvira et al., 2013; Van Lippevelde et al., 2013; Flores-Barrantes et al., 2022).

In this study, parents were asked (Table 2) whether they think their child is overweight, and on average 12.6% answered affirmatively, which does not correspond with the proportion of children classified as overweight or obese, which together amounts to 30.1% (Osmančević-Muminović, 2024). This means that at least 17.5% of parents do not have an accurate perception of their child's weight status. Most parents (93.2%) are aware that being overweight affects health, and 75.5% believe they know what constitutes a proper diet; however, 48.9% feel they need

additional education. On average, 42.4% of parents obtain nutritional information from their pediatrician. A high proportion of parents (92.4%) reported that they would change their child's dietary habits if their child's health were at stake. Parental responses showed no statistically significant differences between urban and rural areas, except for questions 4 and 5. These results highlight the need for parental education.

Table 2. Parents' attitudes toward children's dietary habits

Questions	Answers	Parents (%)		
		Rural	Urban	Total
(1) Do you think your child is overweight?	Yes	12.0 ^{Ba}	13.2 ^{Ba}	12.6
	No	83.0 ^{Aa}	83.2 ^{Aa}	83.1
	I haven't thought about it	5.0 ^{Cc}	3.6 ^{Cc}	4.3
(2) Does being overweight affect health?	Yes	92.9 ^{Aa}	93.4 ^{Aa}	93.2
	No	7.1 ^{Ba}	6.6 ^{Ba}	6.8
(3) Do you know what constitutes a proper diet for your child?	Yes	74.5 ^{Aa}	76.6 ^{Aa}	75.5
	No	25.5 ^{Ba}	23.4 ^{Ba}	24.5
(4) Do you give your child any dietary supplements?	Yes	22.7 ^{Ba}	27.7 ^{Bb}	25.2
	No	77.3 ^{Aa}	72.3 ^{Ab}	74.8
(5) Where do you find advice or information about your child's nutrition?	Internet	37.6 ^{Ba}	47.4 ^{Ab}	42.5
	From a pediatrician	45.4 ^{Aa}	39.4 ^{Bb}	42.4
	From acquaintances	17.0 ^{Ca}	13.2 ^{Cb}	15.1
(6) Would you change your child's dietary habits for the sake of their health?	Yes	94.3 ^{Aa}	90.5 ^{Aa}	92.4
	No	0.7 ^{Ca}	2.9 ^{Ca}	1.8
	I don't know	5.0 ^{Ba}	6.6 ^{Ba}	5.8
(7) Do you feel you need more education about proper nutrition?	Yes	42.6 ^{Ba}	31.4 ^{Ba}	37.1
	No	44.0 ^{Aa}	54.0 ^{Ab}	48.9
	I don't have time for that	13.4 ^{Ca}	14.6 ^{Cb}	14.0

Legend: $^{a, b, c}$ Different letters within rows and columns indicate significant differences ($p \leq 0.05$) based on Duncan's test.

Given that the education system in BiH is decentralized, there is no unified national education system; education is organized at the entity, cantonal, and district levels, which is quite complex and results in significant territorial differences. One common factor, however, is the lack of organized school meals with hot options and the absence of unified guidelines aligned with WHO recommendations regarding physical activity, sedentary behaviour, and dietary habits. While such elements exist in curricula, they are variably detailed across regions. In the Federation of BiH, a Guide for Healthy Nutrition of School-Age Children has been published by the Federal Public Health Institute (PHI FBiH, 2023), and various documents have been issued at different administrative levels. In the USC, there are no guidelines for any of the topics investigated. Therefore, based on the results of this study, the authors propose the following key guidelines to the competent Ministry of Education, in line with WHO recommendations (2006, 2020):

Guidelines for Physical Activity

Children should be physically active for at least 60 minutes daily. The results of this study show that about 61% of children in the USC achieve this level of activity through a combination of school physical education and sports training, which is comparable to data from other European countries. However, around 28% of children are insufficiently active or do not exercise at all, requiring additional interventions. It is recommended to provide children with a minimum of 60 minutes of moderate-to-vigorous activity daily through school and extracurricular programs, increasing opportunities for spontaneous play and movement, and encouraging cooperation between schools and parents to monitor and promote regular activity, with a special focus on children who are physically active less than twice a week.

Guidelines for Sedentary Behaviour

Children's time spent in sedentary activities, especially in front of screens, should be limited, and frequent breaks and active play should be encouraged. Research in Europe shows a wide range of screen time, while in USC, 29.3% of children spend more than 2 hours daily in front of screens, placing USC in the mid-range compared to other countries. Therefore, it is recommended to limit screen time to ≤ 2 hours per day, introduce regular active breaks at school and at home, and support parents and schools in establishing screen-time rules and promoting active play.

Guidelines for Dietary Habits and Daily Meals

Children should eat breakfast regularly, as it has a protective effect on health. The study in USC shows that on average 19.0% of children skip breakfast, which is higher than in some European countries and requires interventions. Nutritiously varied and appropriate school meals should be provided, and the proportion of children bringing meals from home (42.9%) should be increased through a structured strategy until school canteens with hot meals are established, while reducing the consumption of low-quality food from shops. Schools and parents should cooperate to encourage and maintain family meals, aiming to develop healthy eating habits and prevent childhood obesity, and promote the habit of consuming the last meal 2–3 hours before bedtime to support optimal metabolism and sleep.

General Recommendation

The study results highlight that parental education is crucial for shaping healthy eating habits in younger school-age children. Parents influence their child's dietary environment through food choices, rules, and modelling behaviours. Despite high awareness of healthy nutrition and the impact of overweight (93.2%), 48.9% of parents report needing additional education. Public health and education systems should therefore collaborate through joint programs on nutrition, physical activity, and digital hygiene.

Conclusion

The results of this study confirm the need for a comprehensive and coordinated approach to improving physical activity, sedentary behaviour, and dietary habits of younger school-age children and/or more broadly within the education system. It is recommended that the Ministry of Education, Science, Culture, and Sports of the Una-Sana Canton establish standardized school programs to monitor physical activity, sedentary behaviour, and nutrition, and develop educational program interventions targeting children, parents, and teachers. This particularly applies to the need to increase opportunities for daily physical activity and active play in schools, limit sedentary time, and develop a school meal model with hot meals to provide children with nutritionally adequate meals. This study provides valuable insight into the health and dietary behaviours of primary school children in USC, highlighting key areas for action to reduce the risk of developing non-communicable diseases (NCDs) and promote healthier growth and development in children.

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ANTIDIABETIC POTENTIAL OF MICROWAVE-ASSISTED AQUEOUS GRAPE SKIN EXTRACTS FROM SELECTED WHITE TABLE GRAPE CULTIVARS

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Summary

Type 2 diabetes mellitus is one of the fastest-growing metabolic disorders worldwide, increasing the demand for naturally occurring compounds capable of improving postprandial glycaemic control. Grape skins represent an abundant source of biologically active phytochemicals, including polyphenols and other antioxidant constituents, which may contribute to antidiabetic activity. In the present study, the antioxidant capacity and inhibitory activities against *α*-amylase and *α*-glucosidase of aqueous microwave-assisted extracts obtained from the skins of ten white table grape cultivars were evaluated. Grape skin extracts were prepared using microwave-assisted extraction with acidified water as an environmentally friendly extraction solvent. Antioxidant activity was determined by the ferric reducing antioxidant power (FRAP) assay, whereas antidiabetic activity was assessed through inhibition of *α*-amylase and *α*-glucosidase. Significant differences among cultivars were observed for all analysed parameters. Sonja exhibited the highest ferric reducing antioxidant capacity, whereas the strongest inhibition of *α*-amylase and *α*-glucosidase was recorded for Fanny and Sonja, respectively. Interestingly, antioxidant activity did not correspond to enzyme inhibitory activity, indicating that the antidiabetic potential of grape skin extracts cannot be predicted solely from their overall reducing capacity. These findings suggest that the qualitative composition of phytochemicals rather than total antioxidant capacity plays a dominant role in digestive enzyme inhibition. Microwave-assisted aqueous extraction proved to be an efficient green extraction technique for obtaining biologically active grape skin extracts, supporting the utilisation of grape-processing by-products as valuable ingredients for functional foods and nutraceuticals.

Keywords: white table grape; grape skin; microwave-assisted extraction; antioxidant activity; FRAP; *α*-amylase; *α*-glucosidase; functional foods

Introduction

Type 2 diabetes mellitus (T2DM) is one of the most prevalent chronic metabolic disorders worldwide and represents a major public health challenge due to its continuously increasing incidence and numerous long-term complications. The disease is characterised by impaired glucose homeostasis resulting from insulin resistance and/or inadequate insulin secretion, leading to persistent hyperglycaemia. Poor glycaemic control substantially increases the risk of cardiovascular disease, nephropathy, retinopathy and neuropathy, making the prevention and management of postprandial hyperglycaemia an important therapeutic objective (Lu et al., 2024; Rastija et al., 2024).

One of the most widely accepted therapeutic approaches for controlling postprandial blood glucose levels is the inhibition of carbohydrate-hydrolysing enzymes in the gastrointestinal tract. *α*-Amylase initiates the hydrolysis of dietary starch into smaller oligosaccharides, whereas *α*-glucosidase catalyses the final conversion of oligosaccharides and disaccharides into absorbable glucose molecules. Inhibition of these enzymes delays carbohydrate digestion and glucose absorption, thereby attenuating postprandial glycaemic excursions. Clinically approved inhibitors such as acarbose, miglitol and voglibose effectively reduce postprandial blood glucose concentrations but are frequently associated with gastrointestinal adverse effects, including abdominal discomfort, flatulence and diarrhoea, limiting long-term patient compliance (Kalinovskii et al., 2023).

Consequently, increasing attention has been directed towards identifying naturally occurring enzyme inhibitors from plant-derived materials. Numerous studies have demonstrated that polyphenols, flavonoids, phenolic acids, tannins, terpenoids and other phytochemicals may inhibit *α*-amylase and *α*-glucosidase through different mechanisms, including competitive binding to catalytic sites, alteration of enzyme conformation and interaction with substrate-binding residues. Besides their inhibitory activity against digestive enzymes, many of these compounds exhibit pronounced antioxidant properties, reducing oxidative stress that contributes to the development and progression of diabetes and its complications (Sekhon-Loodu and Rupasinghe, 2019).

Grapes (*Vitis vinifera* L.) are recognised as one of the richest dietary sources of bioactive phytochemicals. While grape seeds have traditionally attracted

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considerable scientific interest, grape skins also contain substantial quantities of biologically active compounds, including flavan-3-ols, flavonols, hydroxybenzoic and hydroxycinnamic acids, stilbenes, proanthocyanidins and numerous non-phenolic antioxidants. The concentration and composition of these compounds depend on genotype, environmental conditions, cultivation practices and the stage of berry ripening, resulting in considerable variation among grape varieties. These phytochemicals contribute not only to antioxidant capacity but also to a broad spectrum of biological activities, including anti-inflammatory, antimicrobial, cardioprotective and antidiabetic effects (Zhang et al., 2015; Ishmael et al., 2012; Hrnčić et al., 2019; Mendes et al., 2013).

Interest in grape by-products has markedly increased in recent years owing to the principles of sustainable agriculture and the circular bioeconomy. Grape pomace, consisting predominantly of skins and seeds, represents one of the largest by-products of the wine and grape-processing industries. Rather than being considered agricultural waste, grape skins are increasingly recognised as valuable raw materials for the production of functional foods, nutraceuticals and natural antioxidants because of their high content of health-promoting phytochemicals. The valorisation of these by-products simultaneously contributes to waste reduction and the development of value-added products (Rastija et al., 2024).

Croatia possesses a rich genetic diversity of indigenous grapevine varieties that constitute an important component of its agricultural heritage. Although these varieties are primarily valued for their contribution to regional viticulture and biodiversity conservation, information regarding the biological activity of their grape skins remains limited, particularly for white table grapevine varieties. Characterisation of their antioxidant capacity and enzyme inhibitory activity could therefore provide additional scientific evidence supporting their utilisation as natural sources of bioactive compounds with potential health-promoting applications (Pejić et al., 2010; Vuković et al. 2023).

Therefore, the aim of this study was to evaluate the antioxidant capacity of microwave-assisted aqueous grape skin extracts obtained from selected white table grape cultivars using the ferric reducing antioxidant power (FRAP) assay and to investigate their *in vitro* antidiabetic potential through inhibition of *α*-amylase and *α*-glucosidase. Furthermore, differences among grape varieties were compared in order to identify cultivars with the greatest potential as sources of natural antioxidant and antidiabetic compounds.

Materials and methodology

Preparation of grape skin extracts by microwave-assisted extraction

Grape clusters were harvested from the experimental vineyard of the Faculty of Agrobiotechnical Sciences Osijek located in Mandićevac, Croatia. The skins were manually separated from the pulp immediately after harvest.

Microwave-assisted extraction was performed using an ETHOS X microwave extraction system (Milestone, Sorisole, Italy). Fresh grape skins were extracted with water containing 1 % acetic acid at a solid-to-liquid ratio of 1:5 (1 g fresh skin to 5 mL extraction solvent). The extraction programme consisted of 500 W for 5 min followed by 250 W for 20 min. The obtained extracts were filtered, aliquoted and stored at -20 °C until further analyses.

Chemicals

α-Amylase from porcine pancreas (EC 3.2.1.1), *α*-glucosidase from *Saccharomyces cerevisiae* (EC 3.2.1.20), acarbose, 4-methylumbelliferyl *α*-D-glucopyranoside, 2,4,6-tripyridyl-s-triazine (TPTZ), ferric chloride, ferrous sulphate, sodium phosphate salts, sodium carbonate, sodium hydroxide, soluble starch, 3,5-dinitrosalicylic acid (DNS), dimethyl sulfoxide (DMSO), acetic acid and all other analytical-grade reagents were obtained from commercial suppliers and used without further purification.

Determination of total phenolic content

Total phenolic content (TPC) was determined using the Folin–Ciocalteu spectrophotometric method according to Singleton et al. (1999), with minor modifications. Briefly, an appropriate aliquot of grape skin extract was mixed with distilled water, followed by the addition of 50 µL of 2 M Folin–Ciocalteu reagent. After vortex mixing, 200 µL of sodium carbonate solution (20 %, *w*/*v*) was added, and the reaction mixture was diluted to a final volume of 1 mL with distilled water. The samples were vortex-mixed after each addition of reagent and incubated in the dark for at least 30 min at room temperature. Absorbance was measured at 750 nm using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Gallic acid was used to construct the calibration curve (0–250 mg/L), and the results were expressed as mg gallic acid equivalents (GAE) per litre of extract. All measurements were performed in triplicate, while distilled water was used as the blank.

Determination of antioxidant activity

Antioxidant capacity was determined using the ferric reducing antioxidant power (FRAP) assay based on the reduction of the Fe^{3+}–TPTZ complex to its ferrous form under acidic conditions, according to Benzie and Strain (1996), with minor modifications. The resulting blue Fe^{2+}–TPTZ complex was quantified spectrophotometrically at 593 nm.
Briefly, 20 µL of grape skin extract was mixed with 200 µL of freshly prepared FRAP reagent. Following incubation at 37 °C for 4 minutes, absorbance was measured at 593 nm. A calibration curve was prepared using ferrous sulphate standards, and antioxidant activity was expressed as µmol $FeSO_4$ equivalents per volume of extract. All analyses were performed in triplicate.

Determination of α-amylase inhibitory activity

The inhibitory activity against α-amylase was determined using a colorimetric assay based on the quantification of reducing sugars released during starch hydrolysis. DNS reagent reacts with reducing sugars under alkaline conditions, producing a coloured complex measurable at 540 nm.
Briefly, grape skin extracts were mixed with phosphate buffer and α-amylase solution, followed by pre-incubation at 37 °C for 15 minutes. Soluble starch was subsequently added as substrate and the reaction mixture was incubated for an additional 15 minutes. The reaction was terminated by adding DNS reagent, followed by heating for 10 min at temperatures above 90 °C. After cooling, absorbance was recorded at 540 nm. Each sample was analysed in triplicate, including its corresponding blank to correct for naturally occurring reducing sugars present in the extracts. Acarbose was used as the positive control.
The percentage inhibition of α-amylase activity was calculated according to Equation (1):

$$Inhibition\ (\%) = \frac{A_{control} - A_{sample}}{A_{control}} \cdot 100 \quad (1)$$

Determination of α-glucosidase inhibitory activity

α-Glucosidase inhibitory activity was evaluated using a fluorimetric assay based on the hydrolysis of 4-methylumbelliferyl α-D-glucopyranoside. Following enzymatic cleavage, the released 4-methylumbelliferone was quantified fluorimetrically.
Briefly, α-glucosidase solution was mixed with diluted grape skin extracts and pre-incubated at 37 °C for 15 minutes. The reaction was initiated by adding the fluorogenic substrate and continued for 20 minutes.
Enzymatic activity was terminated with sodium carbonate solution, after which fluorescence intensity was measured using excitation and emission wavelengths of 360 and 450 nm, respectively. All measurements were performed in triplicate. Acarbose served as the positive control.
The percentage inhibition of α-glucosidase was calculated according to Equation (2):

$$Inhibition\ (\%) = \frac{FI_{control} - FI_{sample}}{FI_{control}} \cdot 100 \quad (2)$$

Results and discussion

Total phenolic content

Total phenolic content of grape skin extracts, determined by the Folin–Ciocalteu method and expressed as mg/L gallic acid equivalents (GAE), varied among the investigated varieties (Figure 1.). The highest value was determined for Sonja (343.51 mg/L GAE), which clearly separated this variety from all other samples. The second highest value was observed for Talisman (85.81 mg/L GAE), followed by Original (70.01 mg/L GAE), Fanny (56.13 mg/L GAE), Stefanie (49.90 mg/L GAE) and Palatina (46.95 mg/L GAE). Lower phenolic contents were recorded for Piroška (34.66 mg/L GAE), Primavera (33.62 mg/L GAE), Sophie (32.62 mg/L GAE) and Arkadia (28.87 mg/L GAE).
The pronounced dominance of Sonja indicates a markedly higher accumulation of Folin–Ciocalteu-reactive reducing compounds in its grape skin extract. Since the Folin–Ciocalteu assay is not fully specific for phenolic compounds, the obtained values should be interpreted as the overall reducing capacity of the extract expressed as gallic acid equivalents. Nevertheless, this assay remains widely used for the comparative estimation of phenolic-rich plant extracts and provides a useful first indication of their potential biological activity. Low standard deviation values recorded for all samples indicate good analytical repeatability.
These results confirm that grape skin extracts of white table grapevine varieties differ strongly in their phytochemical potential. Such variation may be attributed to genotype-dependent differences in phenolic biosynthesis, berry skin structure and

accumulation of reducing constituents. In comparison with the previously investigated indigenous Croatian white wine grapevine varieties, where Svetokriška belina showed the highest phenolic content (Rastija et al., 2024), the present results suggest that selected white table grape varieties, especially Sonja, may also represent valuable sources of phenolic and other reducing compounds.

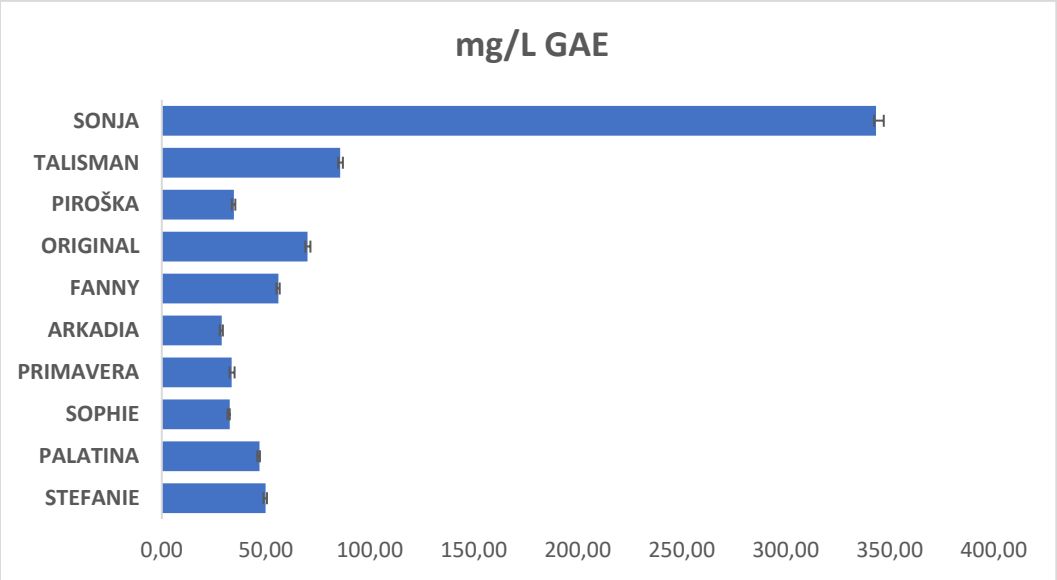

Figure 1. Total phenolic content of grape skin extracts expressed as mg gallic acid equivalents (GAE) per litre of extract. Values are presented as mean ± SD (n = 3)

Ferric reducing antioxidant power (FRAP)

The antioxidant capacity of grape skin extracts was evaluated using the ferric reducing antioxidant power (FRAP) assay, and the results are presented in Figure 2. Considerable differences were observed among the investigated grapevine varieties, indicating marked differences in the reducing capacity of the investigated cultivars.

Among the analysed samples, the Sonja variety exhibited by far the highest antioxidant capacity, reaching 281.15 ± 5.41 μmol Fe^{2+} equivalents g^{-1} fresh weight (FW). This value was approximately three times higher than those recorded for the remaining varieties and clearly distinguished Sonja as the variety with the greatest reducing potential. High FRAP values were also determined for Talisman (95.48 ± 0.30 μmol Fe^{2+} eq. g^{-1} FW) and Arkadia (95.17 ± 0.40 μmol Fe^{2+} eq. g^{-1} FW), followed by Original (87.31 ± 0.65 μmol Fe^{2+} eq. g^{-1} FW), Piroška (79.86 ± 1.61 μmol Fe^{2+} eq. g^{-1} FW), Fanny (74.75 ± 1.95 μmol Fe^{2+} eq. g^{-1} FW), Sophie (73.97 ± 0.58 μmol Fe^{2+} eq. g^{-1} FW), Stefanie (73.43 ± 0.81 μmol Fe^{2+} eq. g^{-1} FW) and Palatina (68.65 ± 1.11 μmol Fe^{2+} eq. g^{-1} FW). The lowest antioxidant capacity was determined for Primavera, with a FRAP value of 53.36 ± 0.97 μmol Fe^{2+} eq. g^{-1} FW.

The relatively low standard deviations obtained for all analysed extracts indicate good repeatability of the analytical procedure and confirm the reliability of the measured antioxidant capacities.

A comparison of the FRAP results with the total phenolic contents determined by the Folin–Ciocalteu assay revealed a similar ranking of grape varieties. The Sonja variety exhibited both the highest total phenolic content (343.51 mg L^{-1} GAE) and the highest ferric reducing antioxidant capacity, suggesting that phenolic compounds substantially contributed to its antioxidant activity.

However, the relationship between total phenolic content and FRAP values was not entirely proportional for all varieties.

For example, Arkadia displayed one of the highest FRAP values despite having the lowest total phenolic content among the investigated varieties, whereas Fanny exhibited relatively high phenolic content but only moderate reducing capacity. Such discrepancies indicate that antioxidant activity depends not only on the total concentration of phenolic compounds but also on their qualitative composition, molecular structure and reducing efficiency. Individual phenolic acids, flavonoids, flavan-3-ols and other reducing constituents differ in their electron-donating ability, meaning that extracts containing similar total phenolic contents may exhibit substantially different antioxidant capacities.

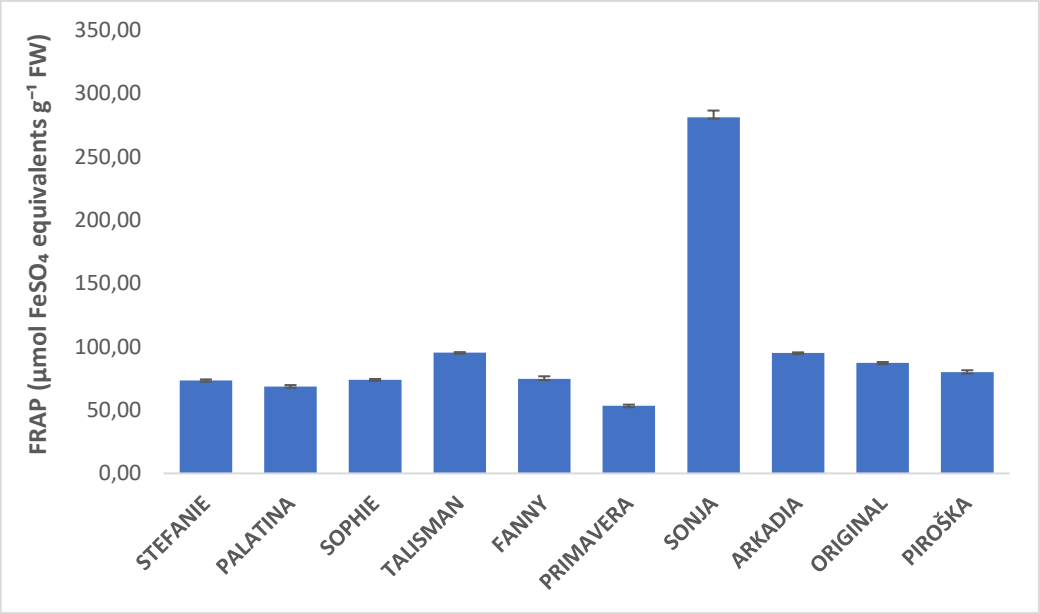

Figure 2. Ferric reducing antioxidant power (FRAP) of grape skin extracts. Values are presented as mean ± SD (n = 3)

α-Amylase inhibitory activity

The inhibitory activity of grape skin extracts against α-amylase is presented in Figure 3. Considerable differences were observed among the varieties, indicating pronounced variability in their ability to inhibit starch hydrolysis.

The highest inhibitory activity was recorded for Fanny, which inhibited α-amylase by 97.64 ± 2.05 %, demonstrating almost complete suppression of enzyme activity. Similarly, the extract of Sonja exhibited strong inhibition (89.17 ± 1.34 %), whereas Original showed moderate to high inhibitory activity (67.34 ± 2.49 %). Intermediate inhibition was observed for Arkadia (57.29 ± 3.52 %) and Palatina (46.29 ± 0.45 %), followed by Piroška (41.57 ± 2.72 %). Lower inhibitory effects were determined for Sophie (30.31 ± 0.45 %), Stefanie (29.43 ± 1.06 %), Talisman (29.52 ± 1.39 %) and Primavera (24.89 ± 2.12 %).

The inhibition of α-amylase by plant-derived polyphenols has attracted considerable attention because delaying starch digestion may reduce postprandial glucose excursions, representing one of the principal therapeutic strategies in the dietary management of type 2 diabetes mellitus.

Interestingly, the present results indicate that α-amylase inhibition was not directly proportional to either total phenolic content or antioxidant capacity. Although Sonja, possessing the highest phenolic content and FRAP value, also showed pronounced enzyme inhibition, the strongest inhibitory activity was observed for Fanny, whose total phenolic content and reducing capacity were moderate. Conversely, Talisman, despite exhibiting one of the highest FRAP values, demonstrated relatively weak inhibition of α-amylase.

These findings suggest that enzyme inhibition depends primarily on the qualitative composition of phenolic constituents rather than their total concentration.

Overall, the results indicate that several indigenous varieties possess considerable α-amylase inhibitory activity, with Fanny and Sonja emerging as particularly promising sources of naturally occurring enzyme inhibitors.

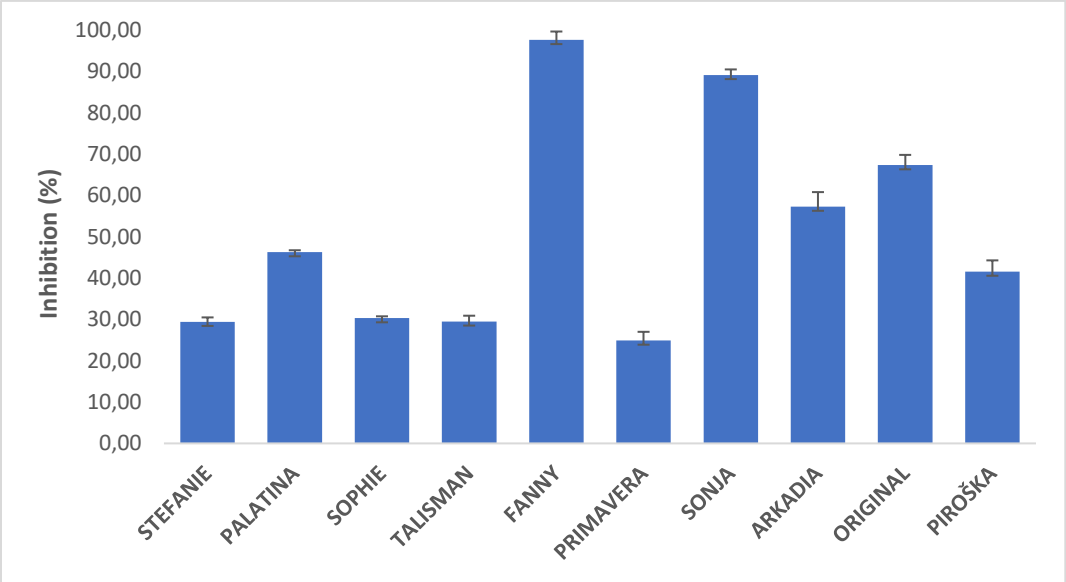

Figure 3. Inhibitory activity of grape skin extracts against *α*-amylase.
Values are presented as mean ± SD (n = 3)

α-Glucosidase inhibitory activity

The inhibitory activity of grape skin extracts against *α*-glucosidase is presented in Figure 4. The highest inhibitory activity was recorded for Sonja, which almost completely inhibited *α*-glucosidase, reaching 100.06 ± 0.12 % inhibition. High inhibitory activities were also observed for Talisman (88.40 ± 0.95 %) and Original (80.32 ± 0.55 %). Moderate inhibition was exhibited by Stefanie (72.93 ± 2.24 %), Primavera (70.52 ± 0.73 %) and Piroška (68.00 ± 0.91 %). Slightly lower activities were recorded for Sophie (63.34 ± 0.90 %), Fanny (61.16 ± 1.61 %) and Arkadia (61.00 ± 0.71 %), whereas Palatina showed the lowest inhibitory activity (58.12 ± 0.60 %).

Despite these differences, all investigated extracts inhibited *α*-glucosidase by more than 58%, indicating a promising *in vitro* antidiabetic potential of grape skin extracts.

Unlike the results obtained for *α*-amylase, the ranking of grape varieties according to *α*-glucosidase inhibition corresponded more closely with their antioxidant characteristics. The Sonja variety, which exhibited the highest total phenolic content and the greatest ferric reducing antioxidant power, also demonstrated the strongest *α*-glucosidase inhibitory activity. Likewise, Talisman and Original, characterised by relatively high FRAP values, ranked among the most effective enzyme inhibitors. These observations suggest that reducing compounds, particularly phenolic constituents, substantially contribute to *α*-glucosidase inhibition.

Nevertheless, the relationship was not entirely linear. For example, Arkadia displayed one of the highest FRAP values but only moderate *α*-glucosidase inhibition, whereas Primavera, despite possessing the lowest antioxidant capacity, exhibited stronger inhibition than several varieties with higher FRAP values. Such observations indicate that enzyme inhibition depends not only on the total concentration of phenolic compounds but also on their structural diversity, molecular interactions and possible synergistic effects among individual phytochemicals.

Comparison of the inhibitory activities against *α*-amylase and *α*-glucosidase further demonstrated that different grape varieties preferentially inhibited individual digestive enzymes. While Fanny exhibited the strongest *α*-amylase inhibition, Sonja proved to be the most potent *α*-glucosidase inhibitor. These findings suggest that different classes of grape phytochemicals may possess different affinities towards the two digestive enzymes, emphasising the complexity of the antidiabetic activity of grape skin extracts.

The obtained results indicate that grape skin extracts of indigenous Croatian white table grapevine varieties possess considerable potential as natural *α*-glucosidase inhibitors. In particular, Sonja, Talisman and Original demonstrated pronounced inhibitory activities and therefore represent promising candidates for further phytochemical profiling and identification of the compounds responsible for their antidiabetic effects.

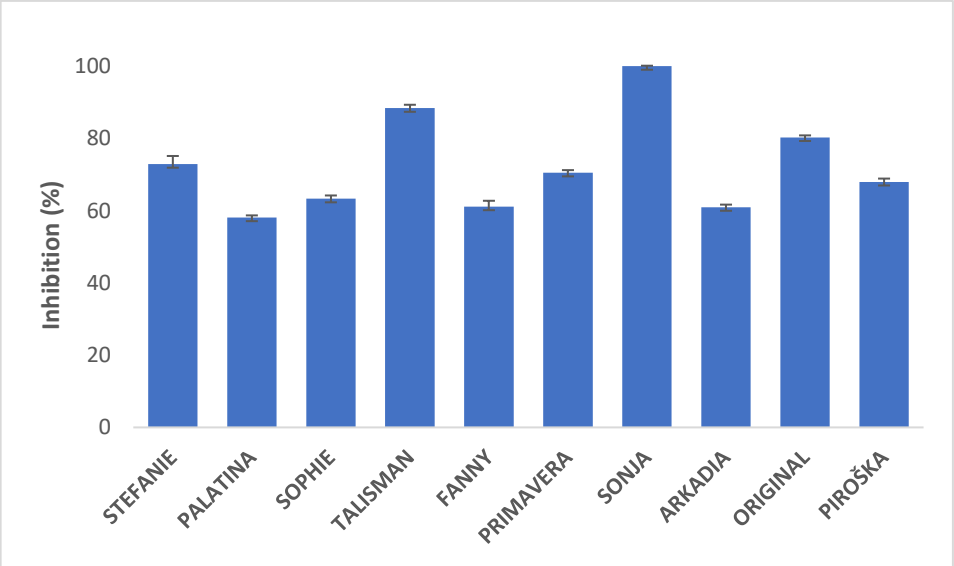

Figure 4. Inhibitory activity of grape skin extracts against α-glucosidase. Values are presented as mean ± SD (n = 3)

Conclusion

The present study demonstrated that grape skin extracts obtained from selected white table grapevine varieties possess considerable antioxidant and *in vitro* antidiabetic potential, although significant differences were observed among the investigated cultivars. The highest total phenolic content, ferric reducing antioxidant capacity and α-glucosidase inhibitory activity were determined for the Sonja variety, indicating that this cultivar represents a particularly rich source of bioactive compounds with potential health-promoting properties. In contrast, Fanny exhibited the strongest α-amylase inhibitory activity, suggesting that the inhibition of carbohydrate-hydrolysing enzymes is influenced not only by the total concentration of phenolic compounds but also by their qualitative composition and possible synergistic interactions among phytochemicals.

The comparison of antioxidant capacity and enzyme inhibitory activities revealed that reducing power alone could not fully explain the observed antidiabetic effects, highlighting the complexity of the phytochemical composition of grape skin extracts. These findings indicate that different grapevine varieties may contain distinct profiles of bioactive compounds that selectively contribute to antioxidant activity and inhibition of digestive enzymes.

These findings support the utilisation of white table grape skins as promising raw materials for functional foods and nutraceuticals with antioxidant and antidiabetic potential while also contributing to the sustainable valorisation of grape-processing by-products.

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FOOD SAFETY THROUGH THE LENS OF PHYTOBIOTICS

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review paper

Summary

In the field of food safety, natural plant compounds are becoming increasingly central to scientific research, and phytobiotics stand out in particular for their numerous functional properties. Phytobiotics comprise various botanical extracts and essential oils. Their importance primarily stems from their bioactive compounds. Their utilization, especially in livestock production, revealed a sequence of positive effects, including reducing pathogenic microorganisms, improving gut microbiota, and decreasing the need for anti-inflammatory drugs and antibiotics. Thus, directly and indirectly contributing to the safety of animal-derived products. Phytobiotics are emerging as an alternative to the traditional additives due to growing concerns for food safety and the need to reduce antimicrobial resistance. Their effectiveness depends on the form and type of plant material, concentration of active ingredients, method of application, and possible interaction with other food components. Simultaneously, a deficit of standardization guidelines, possible presence of contaminants, and limited regulatory clarity are challenges in their wide usage. This review provides an overview of phytobiotics and their impact on animal health, their potential in improving food safety, and the risks associated with improper use. By analyzing the available literature, emphasis is put on the need for additional research, regulatory guidelines, and ensuring quality control.

Keywords: phytobiotics, animal nutrition, food safety

Introduction

Today's intensive meat, milk, and egg production is faced with enormous challenges, such as maintaining animal health, ensuring food safety as well, and protecting consumers from harmful substances and pathogens. One of the major challenges is antimicrobial resistance which is associated with long and non-selective use of antibiotics in animal husbandry, not only for curing illness, but for growth promoters as well. The aforementioned led to the adoption of legal regulations connected to a ban on the use of antibiotics as growth promoters. One of the first countries to prohibit such use of antibiotics was the Netherlands. This resulted in a 51% drop in antibiotics sales (Lykhach and Lykhach, 2023). The ban has been running since 2006 in the European Union (Hashemi et Davodi, 2010; Akyildiz et Denli 2016). Solution is also sought to minimize the use of veterinary antibiotics (Steiner et Syed, 2015). Farmers, veterinarians, researchers, healthcare professionals, and consumers are increasingly concerned about foodborne diseases and their consequences on human health. This is aggravated by the growing resistance of microorganisms to antibiotics and excessive consumption of medical antibiotics (Friedman, 2007). It is the growing concern that has prompted research for alternatives with similar positive effects but without negative side effects (Grashorn 2010, Pandey et al., 2023).

Phytobiotics are defined as products from plants and have positive effects on gastrointestinal microflora, performance, and ultimately on the quality of animal products (Arpášová et al., 2013; Alem et al., 2024). Regarding the rapid utilization of phytobiotics in livestock production and their potential in the sustainability of the food chain, it is crucial to know and accept the mechanisms of their activity, advantages, and limitations in the feed of domestic animals, as well as regulatory requirements. Therefore, this paper aims to present and analyze the role and dynamics of applying phytobiotics in the food of domestic animals, with an emphasis on their effects on healthiness and quality of animal products and potential risks for food safety and consumer health. This paper provides an overview and highlights the importance of a balanced approach to applying phytobiotics in order to gain safe animal-based products.

The role and classification of phytobiotics

Phytobiotics or phytogenic food additives describe a group of bioactive substances of plant origin that are added to the feed of domestic animals to improve growth, health status, production efficiency, and quality of animal-based products (Friedman, 2007; Arpášová et al., 2013). They are natural compounds and can be made from spices, fruits, medical herbs, and parts of plants (Oni et al., 2024). Their primary function is not nutritional, but rather physiological and functional (Abdelli et al., 2021) as they

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are often implemented in animal feeding as alternatives to antibiotics as growth rate promoters (Kiczorowska et al., 2017).

Leaves, roots, flowers, as well as whole plants, are used to produce phytobiotic products. Products may contain dried forms of whole plants or their parts or extracts of valuable ingredients (Grashorn, 2010). Although there aren't any final criteria of classification, based on the origin and characteristics of the processing of phytobiotics, they can be arranged in four categories: spices, herbs, essential oils, and oleoresin (Senčić et al., 2021; Pandey et al., 2023). They can be used in various forms, such as solid, granular, or liquid form. Solid form is usually added to premixes or complete feed, while the liquid form is added usually to drinking water or milk substitute, or can be added by spraying onto hydrothermally processed food (pelletized or extruded food) (Steiner et Syed, 2015). Abdelli et al. (2021) in their research emphasize many spices and herbs, such as thyme, rosemary, oregano, cinnamon, marjoram, yarrow, ginger, garlic, green tea, black cumin, as well as essential oils such as thymol, garlic, anise, cinnamaldehyde, rosemary, citrus, clove, and ginger, used in poultry production. It is worth noting that combinations of different groups are often used to achieve a synergistic effect and increase the stability of formulations during storage and final food processing (Zeng et al., 2015). This is confirmed by the research Alem et al. (2024), in which the authors affirm the synergistic antibacterial effect of plants, for example, against bacteria such as *Staphylococcus spp*., *Streptococcus spp*., *Salmonella spp*., and *E. coli.*

Bioactive components of phytobiotics

Bioactive compounds of phytobiotics include terpenoids, flavonoids, alkaloids, phenols, glycosides, and glucosinolates (Demir et al., 2005; Ivanova et al., 2024). How many bioactive substances and their chemical composition in phytobiotics depend on various factors such as geographical location, part of the plant, and season of harvesting (Huyghebaert et al., 2011; Kiczorowska et al., 2017; Pandey et al., 2023; Alem et al., 2024). With that, herbal extracts also depend on the moisture content of the material, drying method, storage condition, and the production method (Liu et al., 2016; Dilbato Dinbiso et al., 2022). The largest group of secondary plant metabolites is terpenoids. Terpenes of some plants show immunosuppressive, bacteriostatic, and bactericidal properties (Rogošić et al., 2013). Saponins play a role in immune regulation, antitumor activities, and lowering cholesterol, while flavonoid glycosides have a role as antioxidants and anti-inflammatory reducers (Ivanova et al., 2024). Alkaloids have various effects as antimicrobial, anticancer, antiviral, diuretic, analgesic, anticholinergic, antitumor, sympathomimetic, antihypertensive, and antiulcerative (Ivanova et al., 2024). Phenol compounds, such as flavonoids, have a role in antioxidative, cardioprotective, antibacterial, cardio-protective, and anti-inflammatory activities (Jarial et al., 2018; Ivanova et al., 2024). Phytoncides are compounds those plants release to protect themselves from microorganisms or as a repellent effect for herbivores. In addition, it is confirmed that phytoncides show antimicrobial, antibacterial, antifungal properties, and anti-inflammatory and analgesic properties. Therefore, it has the potential to be used as a preserving agent in the food industry (Memon et al., 2021; Ivanova et al., 2024).

Effects of phytobiotics application on animal health and productivity

Beneficial effects of phytogenic compounds come from their bioactive molecules (Yitbarek, 2015), which have a part in antioxidative, antimicrobial, anticoccidial, anthelmintic, and anti-inflammatory response (Gregačević et al., 2014; Alem et al., 2024). They are powerful positive agents of the immune and digestive systems of animals (Akyildiz et Denli, 2016). With that, they improve the digestibility and maintain and stabilize beneficial microflora in the intestine (Placha et al., 2022). They stimulate the microbes in the rumen, additionally the entire digestive tract, and improve the palatability of food (Alem et al.,2024). Furthermore, they affect growth rate, reduce the number of new diseases, advance parameters of reproduction, and have a positive effect on feed efficiency, profitability, and gas emissions when they are used in poultry feeding (Yitbarek, 2015). Besides that, they reduced microbiological load and improved sensory evaluation, leading to better quality of carcass meat (Grashorn, 2010). Furthermore, the usage of phytobiotics as an alternative to antimicrobial medicine can lead to health protection and the improvement of animal productivity, but also to solving the problem of food safety by removing the cumulative effects of antibiotics in animal products (Ryazanov et al., 2021). With that, plant extracts in our meals help reduce oxidative rancidity and maintain the nutritional quality of the animal products (Alem et al., 2024).

Overview of previous research on the influence of phytobiotics on animal welfare, productivity, and the quality of animal products

In the research Al-Beitawi and El-Ghousei (2008), the effect of different quantities of smashed and non-smashed black cumin seeds *(Nigella sativa)* in feed was studied. Results showed that chicks fed with 1.5% smashed cumin seeds had better body weight, body mass gain, and feed conversion ratio. Furthermore, feeding containing 3.0%

cumin seeds (smashed and non-smashed) lowers the concentration of cholesterol and triglycerides in the blood plasma of chicks. Cisse et al. (2025) researched the influence of the citrus extract on animal growth, welfare, and gut microbiota of broiler chicks. Results show improvement in the final body weight and carcass quality of the broiler chickens. In addition, the appearance of foot dermatitis and intestinal health improved. In the research, Giannenas et al. (2005) included 210-day-old chickens. It was confirmed that the inclusion of ground oregano into the diet can reduce the adverse effects of *E. tenella* infection. The authors concluded that oregano could be included in complete broiler feed mixtures as another method to substitute ionophore antibiotics against *E. tenella*. Gumowski et al. (2025) in their research concluded that phytobiotics formulations offer a viable alternative to traditional commercial chemical products that promote the growth rate of poultry, with a positive effect on the performance of the broiler, the health of the gastrointestinal tract, and meat characteristics. The research by Demir et al. (2005) was conducted on broilers. They compared five herbal feed additives (oregano, Du-Sacch, saponin, garlic, thyme) and antibiotics and their effects on growth rate. Results showed that antibiotics can be substituted with natural herbal additives as growth promoters in broilers. Prakatur et al. (2021) conducted research in which they determined the influence of chamomile and nettle on the meat quality of broilers. Results indicated that listed natural supplements effectively affect the pigmentation of the chicken's skin, and this is precisely what makes these products visually more alluring to consumers, which definitely also influences their choices when purchasing such products. Klarić et al. (2019) researched the impact of chamomile and nettle supplementation on biochemical and production parameters of broilers. The conducted research confirmed that nettle and chamomile can be used as ingredients in feed for fattening chicks because positive effects on researched indicators were observed.

Research was carried out to examine the influence of oregano essential oil on the color and oxidative stability of chicken breast meat (raw and cooked). The oregano lowered the protein and lipid oxidation and has a positive effect on the color of raw and cooked meat. The authors believe that oregano essential oil at a concentration of 100-400 ppm have a potential to take the place of synthetic antioxidants in the meat of chicken (Al-Hijazeen et al., 2016). The aim of Iwiński et al. (2023) research was to evaluate consequence of different doses of phytocide mixtures on production and meat quality. Besides that, they studied the possibility of residues and toxic effects on broilers. It was established that the use of phytobiotic supplements does not influence the pathological changes of organs and does not relate to the appearance of residues in tissues.

Duarte and Kim (2022) studied the influence of phytobiotics on growth and intestinal health in pigs. They came to the conclusion that phytobiotics, similar to antibiotic drugs, improve the growth rate from the weaning phase to the fattening phase by acting as inflammatory and oxidative reducers. Furthermore, phytobiotics have positive effects on immune response and consequently the morphology of the jejunum. However, they found that the combined application of phytobiotics with antibiotics suppresses the effects. Lykhach and Lykhach (2023) researched the effects of liquid and dry forms of phytobiotics („Liptosa Expert ") on the growth rate of crossbred young pigs. The use of phytobiotics during the period of birth, rearing, and fattening can be an effective method of substituting antibiotics and growth stimulators, which leads to increased survival rate of piglets during the weaning period, live weights during the fattening period, and the development of beneficial microflora in the pig's intestine. The aim of the research by Katsoulos et al. (2017) was to determine the efficiency of the essential oil of oregano in stopping neonatal diarrhea in young calves (<15 days old) in field conditions. Results throughout the research, loose bowel frequency, duration, and severity were significantly reduced in the experimental group. They concluded that applying the essential oil of oregano to calves during the first 10 days of life reduces the intensity of inherently acquired diarrhea and has a prophylactic effect against neonatal diarrhea. Luo et al. (2024) researched the influence of different doses of oregano essential oil on stimulating the growth of the gastrointestinal tract and improving the immune system in calves. Results indicated that adding oregano oil had a positive effect on weight and average daily growth, as well as rising serum concentration of IgA and IgM, and remarkably increased the number and diversity of rumen microbiota. The goal of the study by Neofytou et al. (2020) was to calculate the effect of ensiled olive scones included in diets on milk production (yield, composition) from cows. By including the olive scones in the diet of cows over a period of 4 weeks, milk composition (lipid profile of milk and Halloumi cheese) was improved without any negative effect on milk production or cows. Winkler et al. (2015) conducted research on 27 dairy cows with the hypothesis that supplementation with an herbal product (95% of green tea and 5% of turmeric extract) reduces inflammation agents and endoplasmic reticulum stress in the liver in early lactation of cows. Although there were small effects related to the occurrence of endoplasmic reticulum stress and inflammation, the authors believe that polyphenol supplementation could be beneficial for improving milk production (yield) and as a prophylactic method for fatty liver disease in dairy cattle. Piran Filho et al. (2021) conducted research with the aim of evaluating the influence of mixed commercial phytobiotics

(„Digestarom“) on the amount of food consumed by animals, feeding behavior, nutrient digestibility, growth rate, and carcass components of bulls. Results show that the phytogenic supplement has a positive influence on feed intake and carcass components without any influence on metabolic disturbances.

A review that included scientific papers published between 2000 and 2020 shows that supplementation of phytogenic additives in the feed of pigs, rabbits, and ruminants advanced product quality in terms of organoleptic properties and fatty acid profile. Furthermore, advanced oxidative stability and storage life (Tsiplakou et al., 2021).

Safety and risks of using phytobiotics

Phytobiotics cannot replace the negative effects of nutrition or nutritional imbalances in meals (Arpášová et al., 2013). They are recognizable by minimal residue levels, absence of resistance growth, and lack of negative impacts (Pandey et al., 2023). Most herbal extracts tested in poultry experiments showed positive effects on productivity and had no adverse impacts on animal welfare as well as animal products (Akyildiz et Denli, 2016). Despite this, the pathway of phytobiotics is indistinct because of the broad spectrum of bioactive matter inclusion in herbal products (Pandey et al., 2023; Alem et al., 2024). Besides that, the slow therapeutic effect and unstable chemical composition make it impossible to accurately calculate the required dose (Pashtetsky et al., 2020). Therefore, special attention should be paid to dose and purity, because in higher concentrations they can have anti-nutritive properties (Almeida et al., 2021). Moreover, production of herbal extracts grapples with many obstacles, including low-volume production, inchoate extraction techniques, indeterminate dosing guidelines (for species, weight, age), expansive costs, and unpredictable effects (Pashtetsky et al., 2020). It is worth emphasizing that supplementation of phytogenic additives in poultry feed in terms of their efficiency and safety is still lacking (Hashemi et Davoodi, 2010; Huyghebaert et al., 2011). Dose adjustment of phytobiotics, better control of their extract production and application, or implementation are core for further research (Obianwuna et al., 2024).

Regulation of the safe use of phytobiotics

Food safety includes measures that ensure that food is safe for the end consumer. It is achieved through legal provisions and private regulation (Knežević et al., 2013). In the EU and our country (Republic of Croatia), the key regulation is Regulation (EZ) no. 1831/2003 on additives in feed. According to this regulation, each additive must be approved by the European Food Safety Authority (EFSA) as well. In our country, the consumption of phytobiotics is additionally regulated by the Food Act (NN 18/2023), which lays down general principles of safety in the „farm to fork “chain. When phytobiotics are used for therapeutic purposes, the provisions of the Veterinary Medicinal Products Act must also be respected.

In the Republic of Croatia, food safety supervision is carried out by various competent institutions, ensuring a comprehensive approach. Different ministries and agencies have specific duties according to manufacturing method and production, and step in the supply chain, which contributes to more efficient risk management (Ministries of Health and Agriculture, Forestry and Fisheries, Croatian Institute for Standardisation) (Knežević et al., 2013).

An important part of the responsible use of phytobiotics is including food safety management procedures and a preventive food safety system known as the HACCP system (Hazard Analysis and Critical Control Points). The implementation of the HACCP system is monitored by sanitary and veterinary inspectors who, in addition to regular inspections of facilities, also monitor hygiene conditions, product labeling, documentation, as well as the presence of biological, chemical, and physical hazards, and ensure traceability throughout the food chain.

Conclusion

Today, phytobiotics have taken up a main position in agriculture, the food production industry, and veterinary medicine, especially in livestock farming. Their bioactive compounds, with many known positive effects, such as antimicrobial, antioxidant, and inflammatory reducers, boost the health of animals and their products. Although they have numerous positive effects, their function and impact depend on many things, such as the form of plant material, the concentration of active ingredients, and the method of application. Improper use or inadequate quality control may increase the risk of contamination or variability in product quality. So, it is necessary to improve regulatory guidelines and the precise determination of doses. Besides that, it is necessary to conduct further research on their long-term effects on animals. To conclude, phytobiotics can improve microbiological stability, product quality, and food safety, but at the same time can be a source of contamination. Therefore, their use needs to be viewed through a prism of an integrated food safety approach, which includes interdisciplinary collaboration among numerous scientists and experts and careful monitoring of each.

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