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FOOD IN HEALTH AND DISEASE

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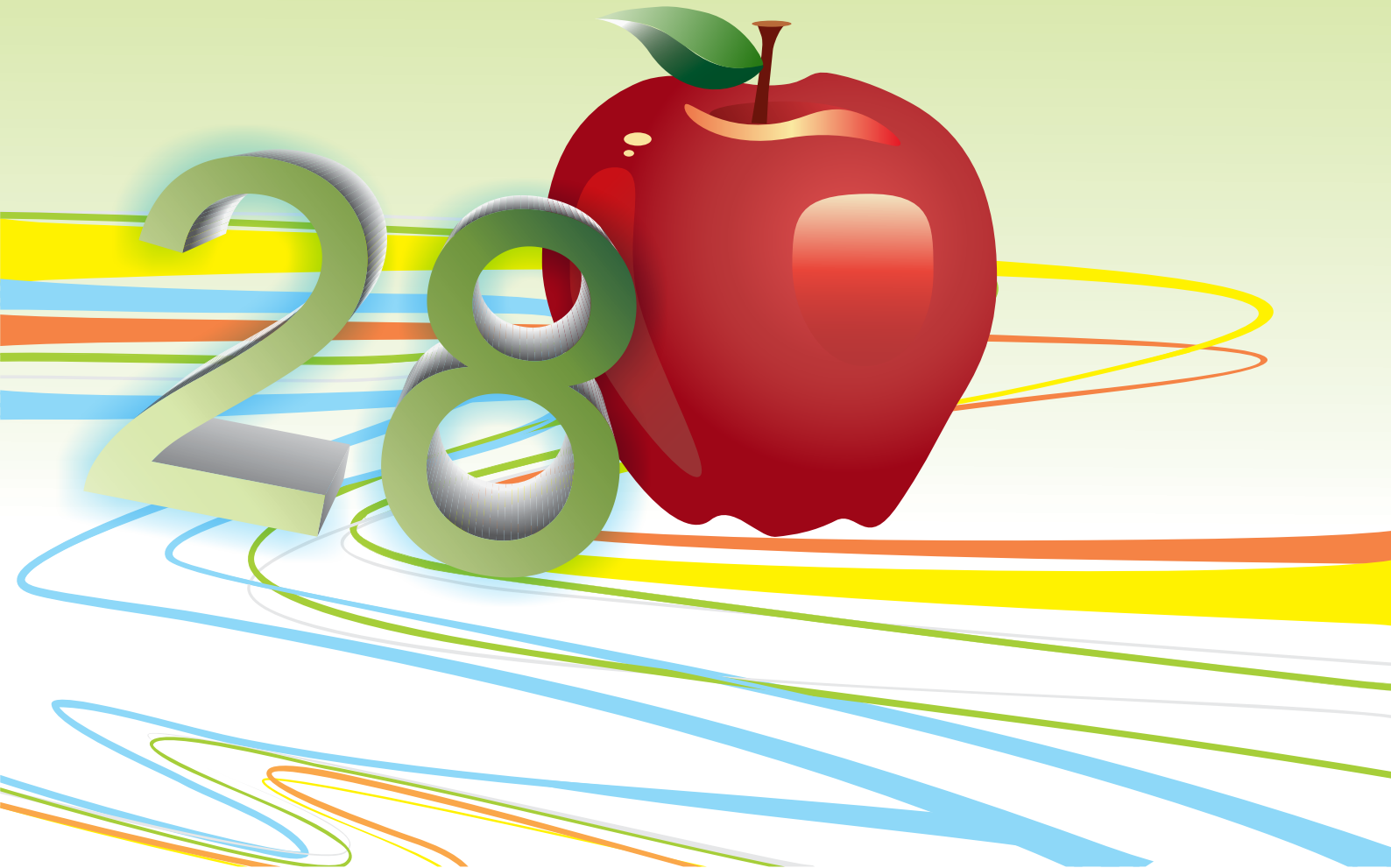

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COMPARATIVE STUDY OF PHYSICAL PROPERTIES OF SOME COMMERCIAL CHOCOLATES IN THE CROATIAN MARKET

Đurđica Ačkar, Patrik El Habbab[#], Antun Jozinović[*], Daniela Paulik, Jurislav Babić, Drago Šubarić

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original scientific paper

Summary

The aim of this research was to investigate colour, texture, and rheological properties of commercial dark and milk chocolates available in the Croatian market. Chocolates were purchased in local stores, and 8 milk chocolate bars and 6 dark chocolate bars, from 5 different producers were obtained. Lightness (L*) of milk chocolates ranged from 33.8 to 41.5, and 29.2 – 33.8 for dark chocolates. Hardness of chocolates determined by breaking test decreased with increasing cocoa solids content, while among milk chocolates, samples M4, which was the thickest, and M5, with the lowest content of milk solids, had significantly higher hardness than all other samples. Casson plastic viscosity ranged between 1.1 Pas and 7.21 Pas for dark chocolates, and 4.2 – 24.79 Pasfor milk chocolates. Since the analysed chocolates are popular among the customers, these values are a good orientation for targeting certain properties when developing new recipes.

Keywords: milk chocolate, dark chocolate, rheology, texture, colour

Introduction

Chocolates are popular confectionery products among all consumer groups – from children to elderly. The variety of products – from dark, milk, white and ruby to different fillings and forms/shapes contributes to high popularity of this treat. According to Statista (2025), chocolate sales averages around 10 billion kilograms, comprising more than 10% of all confectionery sold worldwide (Figure 1). During consumption, chocolate melts in the mouth and flavour is released. The flavour of chocolate is determined both by volatile (alcohols, esters, fatty acids) and fat-soluble non-volatile compounds, and fat and sugar contribute to the sensation. Particle size and flow properties of chocolate are also important for a mouthfeel and acceptability of the products by the consumers and colour is the first quality attribute consumers evaluate when choosing food (Boruczkowska et al., 2025).

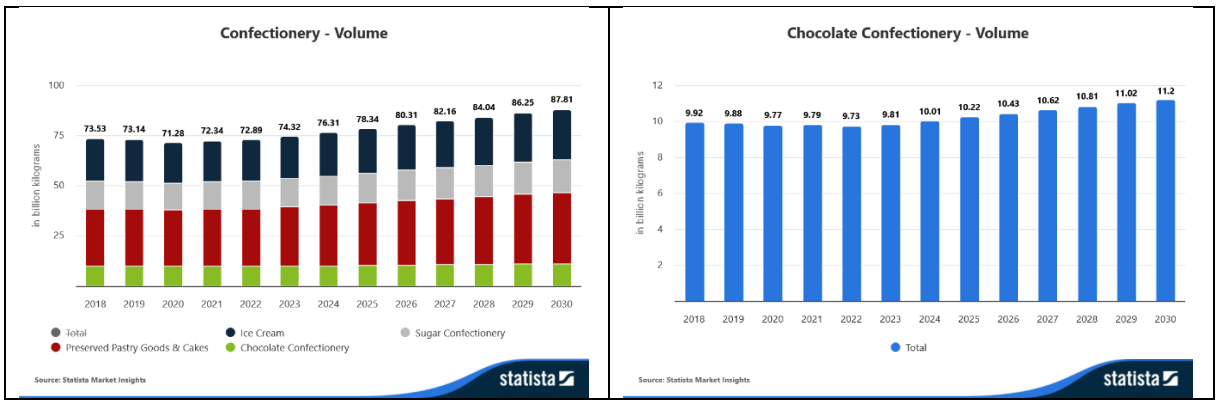

Figure 1. Quantities of confectioneryand chocolate sold worldwide with estimated quantities for future period (Source: Statista, 2025)

In addition to sensory analysis, physical properties of food (chocolate) may be assessed by analytical tools. For instance, colour of chocolate may be evaluated by sensory analyst, but it can also be measured by chromameter, which gives results comparable to human eye, mimicking perception of the eye through registering three primary colours: red, green and blue. In the food industry chromameters are often used, despite their limitations when colour is dependent on texture and turbidity, and CIELab system is commonly employed. This is a three-dimensional system in which lightness (L), green to red (a*) and

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blue to yellow (b*) colour components are placed in a coordinate system, giving the colour of the sample (Boruczkowska et al., 2025).

Texture is another physical property important from sensory perspective. Although Civille and Seltsam (2014) claim that instruments cannot mimic texture sensation in the mouth and that texture can only be evaluated by humans, texture analysers are often used in the food industry. They give the advantage of repeatability, although they do not mimic overall texture sensation in the mouth (biting force, mastication, sound…). Force instruments apply puncture, compression–extrusion, cutting–shear, compression, tensile, torsion, bending and snapping, or deformation, depending on their configuration (Bourne, 2002) and give insight into forces needed to break, chew or masticate the food sample, and/or its gumminess and adhesiveness.

Perhaps most important physical property of chocolate in industrial level is rheology. Flow properties are important not only from sensory aspect, but for handling chocolate during production – pumping, piping, moulding, coating. Standard ICA (International Confectionery Association) method refers to rheological measurements by rotational viscometerwith concentric cylinders at 40 °C and use of Casson model to calculate Casson plastic viscosity and Casson yield stress (Goncalves and Lannes, 2010). Plastic viscosity is important for pumping, coating, filling rough surfaces, and mouthfeel. High-viscosity chocolates have pasty mouthfeel and stick in mouth for longer period of time. Yield stress is a transition point between pseudo-solid and pseudo-liquid state and shows minimum force needed to force chocolate to flow.

The aim of this research was to investigate colour, texture and rheological properties of commercial dark and milk chocolates available in the Croatian market in order to examine acceptable ranges of instrumental analyses results that could be used as a basis for targeted properties when developing new recipes.

Materials and methods

Chocolates were purchased at convenience stores in Osijek, Croatia, as chocolate bars of 75 – 100 grams, depending on the availability. Total of 8 milk chocolate bars and 6 dark chocolate bars, from 5 different producers were obtained, with specifications shown in the Table 1. Dark chocolates D1 and D2 were labelled as chocolates for cooking and eating, and chocolates M6, D3 and D6 were labelled as high-protein chocolates.

Table 1. Specifications of commercial chocolates, available in the Croatian market, used in the research

CHOCOLATE	COCOA SOLIDS (%)	MILK SOLIDS (%)	TOTAL FAT (%)	MILK INGREDIENTS	SPECIFIC INGREDIENTS	BAR MASS (g)
M1	25		32.3	skimmed milk powder whey powder, milk fat	palm, shea hazelnut paste	80
M2	30	23	30.4	whole milk powder, skimmed milk powder		75
M3	30	23	30.4	whole milk powder, skimmed milk powder		75
M4	31	7	34.0	whole milk powder		100
M5	31	18	32.9	whole milk powder, whey powder		80
M6#	32	19	35.0			80
M7	33		31.0	skimmed milk powder whey powder, milk fat	hazelnut paste	80
M8	40		33.0	skimmed milk powder milk fat		85
D1*	43		27.7		palm, shea	100
D2*	43		29.0			100
D3#	55		35.0		whey protein	80
D4	60		38.0			100
D5	72		42.1			100
D6#	74		38.5		dietary fibre (8%), milk protein (18%)	80

*Chocolates for cooking and eating; #high-protein chocolates

Chocolates were stored in the original packaging at ambient temperature until analyses, but no longer than one week.

Immediately after opening, **colour** of the bottom surface (which is flat, without markings/ornaments and crevices) was determined using Chroma Meter C-400 (Konica Minolta), in CIEL*a*b* and L*Ch systems. Prior to measurement, the instrument was calibrated using white tile. Obtained parameters were:

- L* - lightness (from 0 – black to 100 – white)
- a* - positive values mark red domain, and negative mark green
- b* - positive values are yellow and negative are blue
- C – Chroma, colour intensity (positive values –brighter; negative – duller)
- h° – hue, colour tonality (from 0° - red to 90° - yellow, 180°- green and 270°- blue).

These colour systems were chosen because they reflect the perception of colour by human eye. The difference between the two system is that L*a*b* has rectangular, and L*C*h cylindrical coordinates.

Texture properties were determined using Texture Analyser TA.XT (Stable MicroSystems, Great Britain), using breaking method (Figure 2a) (Three Point Bend Rig)and by penetration (Figure 2b) (heavy-duty platform and 2 mm cylinder probe). Hardness and brittleness values were obtained using integrated software of the texture analyser.

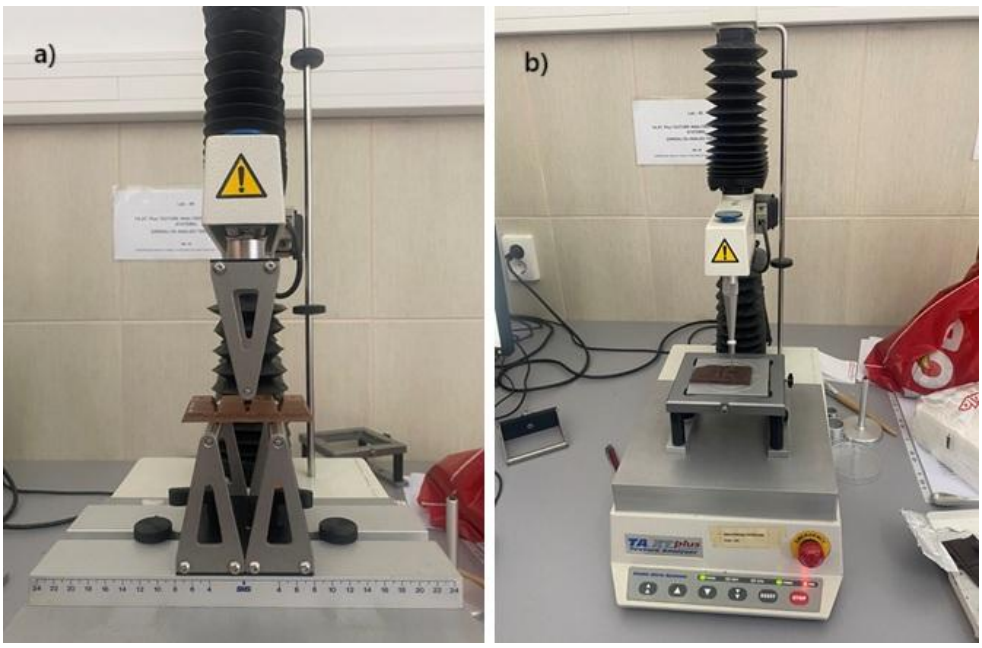

Figure 2. Texture analysis of chocolate samples by breaking test (a) and penetration test (b)

Rheological properties were determined using rotational rheometer HAAKE ViscotesteriQ (Thermo Fisher Scientific, USA) and IOCCC method (2000), at 40 (±0.1) °C. Prior to measurement, samples were thermostated at 50 °C in the water bath, and liquid chocolate was transferred to the measuring cup of the rheometer. Samples were homogenised at 40 °C for 5 minutes at rotational speed of 5 s^{-1}, after which the rotational speed was gradually increased to 50 s^{-1} over period of 180 s, kept at 50 s^{-1} for 60 s and gradually decreased to 2 s^{-1} over period of 180 s.

Rheological parameters were determined through Casson model, where:

- $\tau_{0,}$ Casson yield stress (Pa)
- μ_p, Casson plastic viscosity (Pas),

and the values were obtained using integrated software of the rheometer.

Statistical analysis was performed using Excel, andStatistica® 14.0.0.15Breakdown & one-way ANOVA LSD test ($p < 0.05$) and Correlation matrices ($p < 0.05$).

Results and discussion

Chocolate colour is the first attribute consumers notice when consuming the product. Typically, they attribute darker colour to higher content of cocoa, and, when consuming milk chocolate, lighter brown colour suggests higher contents of milk components. Indeed, chocolate colour is mainly influenced by the content of non-fat cocoa solids and, for milk chocolates, by the content of dairy ingredients (milk powder, whey). However, L* values shown in the Table 2, do not reflect the trend of the increase of the cocoa solids or the milk solids contents. Namely, among milk chocolates, after the sample M8, with the highest cocoa solids content of 40%, sample M1, with the lowest declared content of cocoa solids (25%) had the lowest L* value (37.66), followed by samples with 30% cocoa solids and 23% milk solids (M2 and M3), sample with 31% cocoa solids and 18% milk solids (M5), sample with 33% cocoa solids and finally, sample with 32% cocoa solids.

There is no observed correlation between lightness of milk chocolates and cocoa or milk solids content (Table 3). Chroma (C) values followed the order:M8 <M7 < M2 < M3 <M1 < M4 < M5 < M6. Although this order better reflects the cocoa solids contents shown through correlation in Table 3, there is also no linear trend.

All measured a* values of milk chocolates are in positive domain, ranging from 8.52 for sample M8 to 10.55 for sample M1. Higher a* values correspond to more pronounced redness of the samples, which should correlate to cocoa solids content. However, the present research does not reveal this trend, similar to lightness and chroma values.

L* values observed for dark chocolates are more in line with cocoa solids contents, however the trend is not strictly linear. Just like milk chocolates, redness (a*) of dark chocolates cannot be correlated to the cocoa solids content (Table 3), hence, the sample with 72% cocoa solids had the lowest a* value and the sample with 74% cocoa solids the highest one among dark chocolates.

Milk chocolates had more pronounced yellow component (b*) than dark chocolates, which contributed to their light brown colour, characteristic for this chocolate type. Hue (h) angle places all samples in the domain of red to yellow.

Colour of chocolate is not influenced only by the contents of ingredients, but on the cocoa roasting conditions prior to use in chocolate as well. Cocoa beans may or may not undergo moisture treatment before roasting, final roasting temperature usually ranges between 120 °C and 140 °C, however it may be below 100 °C in some cases, and roasting time may vary as well (Becket et al., 2017). During roasting, Maillard and Amadori reactions may proceed to different extent, giving different compounds such as pyrazines, methylxanthines, melanoidins, acrylamides, biogenic amines, and polycyclic aromatic hydrocarbons (PAHs) (Konar et al., 2025), resulting in different extent of brown colour formation, which, in turn, will influence the colour of the final product. In addition, during storage fat bloom may occur, increasing the lightness due to formation of thin layer of cocoa butter on the surface (Indiarto et al., 2025).

Therefore, colour of chocolate is a complex result of chocolate composition, production and storage conditions, not sole results of ratio of ingredients.

This is further supported by the fact that lightness of milk chocolates in this research is higher than lightness of vegan milk chocolates in research of Indiarto et al. (2025), with a* and b* values in the similar range, and lightness of dark chocolates is higher than the values observed by Kamali et al. (2025) for dark chocolate and dark chocolates with the addition of oleogels, with lower b* values, and a* in comparable range.

Table 2. Colour parameters of selected milk (M) and dark (D) commercial chocolates determined by chroma meter

SAMPLE	L*	a*	b*	C	h°
M1	37.66 ± 0.34^{e}	10.55 ± 0.14^{i}	10.76 ± 0.24^{h}	15.23 ± 0.13^{k}	45.94 ± 0.30^{h}
M2	39.37 ± 0.21^{f}	9.34 ± 0.11^{g}	10.50 ± 0.05^{g}	14.04 ± 0.07^{i}	48.38 ± 0.23^{j}
M3	39.47 ± 0.32^{f}	9.29 ± 0.88^{g}	10.77 ± 0.16^{h}	14.48 ± 0.19^{j}	48.34 ± 0.09^{j}
M4	41.43 ± 0.07^{h}	9.71 ± 0.16^{h}	11.78 ± 0.34^{h}	15.31 ± 0.20^{k}	50.95 ± 0.21^{k}
M5	39.68 ± 0.07^{f}	10.40 ± 0.15^{i}	11.51 ± 0.13^{i}	15.59 ± 0.17^{l}	47.63 ± 0.20^{i}
M6	41.52 ± 0.33^{h}	10.26 ± 0.01^{i}	12.64 ± 0.18^{i}	16.22 ± 0.13^{m}	50.60 ± 0.51^{k}
M7	40.69 ± 0.26^{g}	9.79 ± 0.06^{h}	9.28 ± 0.10^{h}	12.69 ± 0.31^{h}	44.54 ± 0.48^{g}
M8	33.82 ± 0.28^{d}	8.52 ± 0.15^{f}	6.21 ± 0.11^{f}	10.56 ± 0.17^{g}	35.91 ± 0.45^{f}
D1	$30.39 \pm 0.20^{b,c}$	7.56 ± 0.08^{d}	4.67 ± 0.07^{d}	8.91 ± 0.05^{d}	31.48 ± 0.24^{c}
D2	30.65 ± 0.20^{c}	7.95 ± 0.10^{e}	4.73 ± 0.24^{e}	9.33 ± 0.19^{e}	31.41 ± 0.38^{c}
D3	30.19 ± 0.21^{b}	6.83 ± 0.08^{c}	4.55 ± 0.10^{c}	8.21 ± 0.11^{c}	33.68 ± 0.38^{e}
D4	29.29 ± 0.06^{a}	6.39 ± 0.17^{b}	2.86 ± 0.08^{b}	6.86 ± 0.03^{b}	24.50 ± 0.45^{b}
D5	29.31 ± 0.30^{a}	5.33 ± 0.05^{a}	1.66 ± 0.07^{a}	5.59 ± 0.03^{a}	17.57 ± 0.49^{a}
D6	30.98 ± 0.49^{c}	8.31 ± 0.05^{f}	5.60 ± 0.16^{f}	10.02 ± 0.17^{f}	32.48 ± 0.45^{d}

Values with different superscripts in the column are statistically different ($p < 0.05$)

Table 3. Correlations between colour parameters and cocoa- and milk solids contents in analysed chocolates (marked values are significant at p<0.05)

	L*	a*	b*	C	h°
L*	1.000000				
a*	0.418898	1.000000			
b*	0.876659	0.766159	1.000000		
C	0.740722	**0.889371**	**0.965103**	1.000000	
h°	**0.948965**	0.116654	0.692562	0.496065	1.000000
cocoasolids	0.818780	0.832744	**0.987209**	**0.968964**	0.613505
milksolids	-0.702538	-0.251415	-0.476681	-0.421150	-0.669951

Another physical property of chocolate influencing consumers' perception is texture (hardness and brittleness). This property is a result of chocolate composition: cocoa solids content, cocoa butter content, type and amount of substitute fats, and thickness of chocolate bar. Hardness is a measure for mechanical resistance of a material to deformation, and in texture analysis it is defined as "the peak force during the first compression cycle" (Sahin and Sumnu, 2006). The results of hardness determined by texturometer are presented in Figures 3 and 4. Hardness of chocolates determined by breaking test (Figure 3) decreased with increasing cocoa solids content, with the exception of D3 and D6 samples. D3 sample was an 80g-bar, which is thinner than 100 g-bars, while D6 contained fibre, unlike other samples and these probably influenced the results. Diaz et al. (2025) reported that the addition of dehydrated blackberry and blueberry pulp reduced, while goldenberry increased hardness of dark chocolate. Although the observed effect cannot be linked to the fiber contents of added pulps (fresh blackberry and goldenberry contain 5.2 – 5.3 fibre/100 g fruit, and blueberry has 2.4 g fibre/100g (Kaume et al., 2011; Antibarro-Ortega et al., 2025; Duralija and Konjević, 2022)), it is obvious that the addition of fruit/fibre indeed causes changes in texture of chocolate.

Among milk chocolates, samples M4 and M5 had significantly higher hardness than all other samples. Sample M4 is significantly thicker than other samples and sample M5 had the second lowest amount of milk solids. Hardness by penetration separates chocolates for cooking (D1 and D2) from other dark chocolates (D3 to D6). Among D3 to D6 chocolates, hardness by penetration (Figure 4) increased with increase of cocoa solids content, with the exception of D6 sample, which contained added fibre. Among milk chocolates, the samples with lower contents of milk solids (M6 – M8) had lower hardness by penetration than samples with higher content of milk solids (M1 – M3). Sample M4 had the highest hardness by penetration, in line with the results obtained by breaking test, again, most probably due to significantly higher thickness. Correlation analysis revealed a negative correlation between hardness determined by breaking test and milk solids, but no other correlation with composition of chocolate was established. On the contrary, Abdolmaleki et al. (2025) observed negative correlation between the maximum force index (hence, hardness) and fat content in chocolate samples. However, they produced chocolate samples in the laboratory, and they had more consistent samples, with controlled changes of composition. Since each producer uses different recipes, different types of fats, different ingredients, it is very hard to reveal the exact component that had the most pronounced effect on texture.

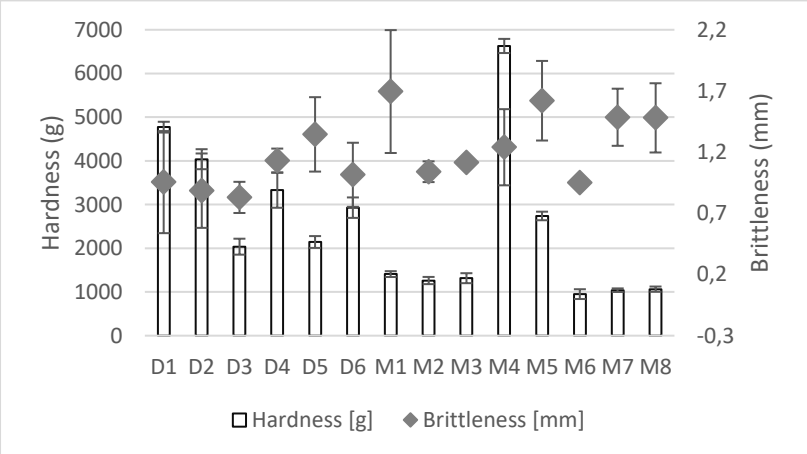

Figure 3. Texture properties of commercial dark (D) and milk (M) chocolates determined by breaking test

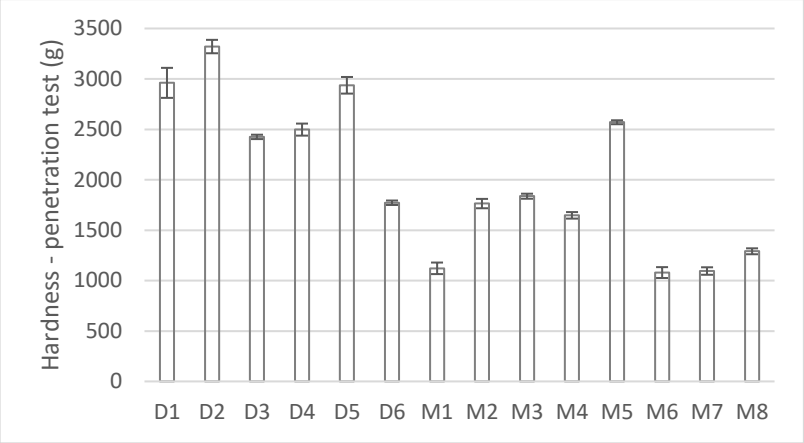

Figure 4. Texture properties of commercial dark (D) and milk (M) chocolates determined by penetration test

Table 4. Correlations between texture properties, cocoa- and milk solids, and fat contents in analysed chocolates (marked values are significant at p<0.05)

	Hardness	Hardness(pen)	Brittleness
Hardness	1.000000		
Hardness(pen)	0.134413	1.000000	
Brittleness	0.385835	**0.896618**	1.000000
Cocoasolids	0.120268	-0.415695	-0.013723
Milksolids	**-0.51613**	0.069000	-0.279421
Total fat	0.378406	-0.385888	0.054433

Liquid chocolate is a suspension of solid particles in fat and its viscosity is influenced by fat contents, size and shape of the suspended particles. Rheological properties of chocolate are important not only for the sensory acceptance, but for handling chocolate during production as well. Chocolate viscosity determines its appearance, texture, mouthfeel and flavour (Becket et al., 2017). Figure 5 and Table 5 reveal characteristic non-Newtonian shear-thinning flow of investigated chocolates. The highest viscosity was observed for milk chocolate M5, followed by dark chocolates for cooking (D1 and D2) and milk chocolate M6. Dark chocolate D5 had the lowest viscosity, whereas all other samples had similar curves (Figure 5). Free fat present in a chocolate largely determines flow properties of chocolates –as the free fat content increases, solid particles become more and more distant and viscosity drops (Beckett et al., 2017). However, part of the fat is bound by and within solid particles and therefore the results in this research cannot be correlated to the total fat content. In addition, particle size and shape influence viscosity as well. Larger number of smaller particles have larger surface area that needs to be covered by fat, whereas large particles may pack together or bound fat. Irregularly shaped particles have to re-align in order to facilitate flow (Beckett et al., 2017). All of these contribute to Casson yield stress, which represents the force needed to start chocolate flow. The highest yield stress was observed for milk chocolate M5, while the lowest was observed for dark chocolate D1. Chocolates D5, M2 and D6 had similar yield stress as well D3, M4 and D4; and M7, M6 and M8.

Dark chocolates had Casson plastic viscosity between 1.1 Pas (D5) and 7.21 Pas (D1), which is comparable to the viscosity of dark chocolates in the research of Sedaghati et al. (2025) who reported Casson plastic viscosity 2.39 – 2.53 Pas, and the research of Thilakarathna et al. (2025), who reported 5.60 –7.90 Pasfor dark chocolates. Plastic viscosity reflects the energy required to sustain fluid flow (Thilakarathna et al., 2025) which is very important for production processes such as pumping, moulding, enrobing, shell making, weight control (Servais et al., 2004) and neither too low or too high viscosities are desirable, whereas very high viscosities will reflect in negative sensory properties, due to sticky and pasty mouthfeel (Castro-Alayo et al., 2023).The quality of raw materials, use of CBEs, sugar substitutes, addition of dietary fiber, bioactive components etc. reflect in

plastic viscosity changes and care should be taken when developing recipes. For example, CBEs with higher contents of saturated long-chain fats will form stable crystals which will increase viscosity of the chocolate, while CBEs rich in medium-chain fatty acids tend to form less stable crystal which will lead to lower viscosity values (Sedaghati et al., 2025). D1 sample in the present research has palm and shea fats, rich in long-chain saturated fatty acids, labelled at the packaging and the highest plastic viscosity value among all investigated chocolates, which is in line with the previous statement. On the other hand, sample M1 had labelled palm, shea, and hazelnut paste and the second lowest plastic viscosity. Hazelnut oil is rich in unsaturated fatty acids (Sun et al., 2022) and probably, although long-chain, these fatty acids decrease crystalline stability and reduce viscosity. In addition, milk fat, cocoa butter and all other components of chocolate mass may interact differently and result in unique viscosities. This is probably the reason for no established statistical correlations (results therefore not shown).

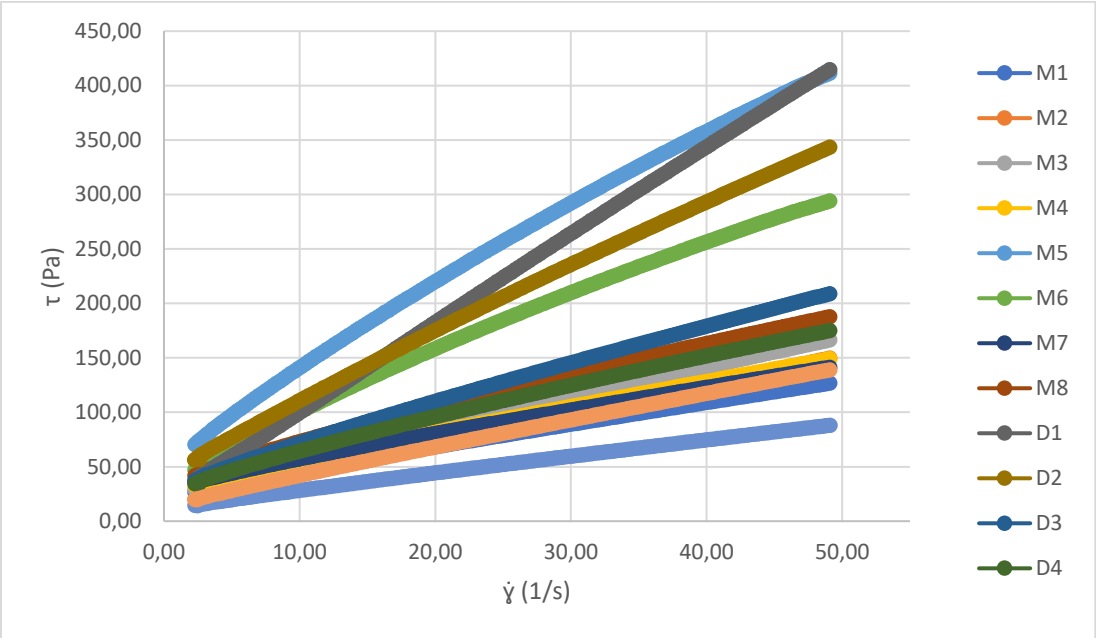

Figure 5. Flow properties of investigated commercial milk (M) and dark (D) chocolates

Table 5. Casson yield stress (τ_0) and Casson plastic viscosity (η_p) of investigated commercial milk (M) and dark (D) chocolates

CHOCOLATE	τ_0 (Pa)	η_p(Pas)	r
M1	10.1 ± 0.13	1.32 ± 0.01	0.9998 ± 0.0001
M2	4.21 ± 0.3	2.09 ± 0.08	0.9997 ± 0.0001
M3	9.22 ± 0.72	1.96 ± 0.02	0.9998 ± 0.0001
M4	13.75 ± 0.75	1.45 ± 0.01	0.9996 ± 0.0002
M5	24.79 ± 0.83	4.85 ± 0.31	0.9999 ± 0.0001
M6	17.34 ± 0.02	3.51 ± 0.13	0.9997 ± 0
M7	16.6 ± 0.76	1.22 ± 0.01	0.9996 ± 0
M8	18.85 ± 0.31	1.78 ± 0.02	1 ± 0.0001
D1	2.39 ± 0.37	7.21 ± 0.03	0.9999 ± 0
D2	16.23 ± 0.47	4.28 ± 0.01	0.9999 ± 0
D3	13.02 ± 0.45	2.39 ± 0.02	0.9999 ± 0
D4	14.15 ± 0	1.83 ± 0.03	1 ± 0
D5	4.06 ± 0.16	1.1 ± 0	0.9997 ± 0
D6	4.69 ± 0.23	1.88 ± 0.02	0.9999 ± 0

Conclusions

Chocolate is a complex system of cocoa parts, milk compounds, sugars, often with added substitute fats and minor ingredients such as emulsifiers and aroma. Therefore, it is hard to predict its physical and sensory properties such as colour, texture, melting and flow properties. Since different ingredients may interact in different manner, depending on their quality, quantity and physico-chemical properties, care must be taken when developing recipes in order to produce a product with desirable technical and sensory characteristics. The results of present research give proper insight in ranges that can be targeted when working on new recipes.

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References

Abdolmaleki, F., Daneshinya, M., Maleki, H. M. (2025): Calorie content, physicochemical properties, and textural characteristics of dark chocolate enriched with date pulp and sesame fiber, *Food Sci. Preserv.* 33(2), 218-231.

Antibarro-Ortega, M., Dias, M. I., Petrović, J., Mandim, F., Nunez, S., Soković, M., Lopez, V., Barros, L., Pinela, J. (2025): Nutrients, phytochemicals, and in vitro biological activities of golden berry (*Physalisperuviana* L.) fruit and calyx, *Plants*14, 327.

Becket, S. T., Fowler, M. S., Ziegler, G. R. (2017): Beckett's Industrial Chocolate Manufacture and Use. John Wiley and Sons Ltd, West Sussex, UK.

Boruczkowska, H., Boruczkowski, T., Bronkowska, M., Prajzner, M., ElzbietaRytel (2025): Comparison of colour measurement methods in the food industry, *Processes* 13, 1268. https://doi.org/10.3390/pr13051268

Bourne, M.C. (2002): Food Texture and Viscosity. *In:* Principles of Objective Texture Measurement, Elsevier.

Castro-AlayoE. M., Baleazar-Zumaeta, C.R., Torrejon-Valqui, L., Medina-Mendoza, M., Cayo-Colca, I. S., Cardenas-Toro, F. P. (2023): Effect of tempering and cocoa butter equivalents on crystallization kinetics, polymorphism, melting, and physical properties of dark chocolates, *LWT – Food Sci. Technol*. 173, 114402.

Civille, G. V., Seltsam, J. (2014): Descriptive analysis of food texture: advances in the sensory characterization of food textures. *In:* Food Texture Design and Optimization, 1st Ed. (Dar, Y. L., Light, J. M., Eds.). John Wiley & Sons, Ltd. pp 321-341.

Diaz, K., Quispe-Sanchez, L., Baleazar-Zumaeta, C. R., Oblitas, R. C., mori, R., Mori, S., Chuquizuta, T. S., Oliva, M., Chavez, S. G. (2025): Rheological, bioactive and sensory properties of dark chocolates elaborated with stevia sweetener and freeze-dried berries, *Appl. Food Res.* 5, 100870.

Duralija, B., Konjević, N. (2022): Blueberry mineral fruit composition, *Pomologia Croatica* 26, 1-4.

Goncalves, E. V., and Lannes, S. C. da S. (2010): Chocolate rheology, *Cienc. Tecnol. Aliment. Campinas* 30(4), 845-851.

Indiarto, R., Al-Amin, R. W., Djali, M., Subroto, E., Muhammad, D. R. A., Wiguna, B. (2025): Improving the properties and sensory acceptability of vegan chocolate formulated with oat milk and soy protein isolate by incorporating encapsulated strawberry extract, *Appl. Food Res.* 5, 100674.

Kamali, E., Sahari, M. A., Barzegar, M., Gavlighi, H. A. (2025): Effect of transesterified amaranth oil oleogel as a cocoa butter replacer on the physicochemical properties of dark chocolate, *Food Chem. X* 26, 102305.

Kaume, L., Howard, L. R., Devareddy, L. (2011): The blackberry fruit: a review on its composition and chemistry, metabolism and bioavailability, and health benefits, *J. Agric Food Chem* 60, 5716-5727.

Konar, N., Palabiyik, I., karimidastjerd, A., Toker, O. S., Gorgulu, A. (2025): Recent advances in cocoa chemistry and roasting, *Trends Food Sci Technol* 162, 105036.

Sahin, S., Sumnu, S. G. (2006): Rheological properties of food. *In:* Physical Properties of Foods. Springer Science+Business Media, LLC, New York, USA. pp. 39-106.

Sedaghati, S., Rajabi, H., Ahmadi, J. (2025): Fortification of dark chocolate with co-encapsulated saffron extract and caffeine, *Appl. Food Res.* 5, 101358.

Servais, C., Ranc, H., Roberts, I. D. (2004): Determination of chocolate viscosity, *J. Texture Stud.* 34, 467-497.

Statista (2025). Market Insights – Confectionery. https://www.statista.com/outlook/cmo/food/confectionery-snacks/confectionery/worldwide (accessed: 29. 9. 2025.)

Sun, J., Feng, X., Lyu, C., Zhou, S., Liu, Z. (2022): Effects of different processing methods on the lipid composition of hazelnut oil: a lipidomics analysis, *Food Sci. Hum. Wellness* JJ, 427-435.

Thilakarathna, R. C. N., Siow, L. F., Tang, T.-K., Cheong, L.-Z., Lee, Y.-Y. (2025): Mahua oil fraction: a sustainable and functional 1,3-dipalmitoyl-2-oleoyl-glycerol (POS)-enriched cocoa butter equivalent for chocolate production, *Food Chem.* 477, 143564.

Hrana u zdravlju i bolesti, znanstveno-stručni časopis za nutricionizam i dijetetiku (2025) 14 (2) 68-74
Food in Health and Disease, scientific-professional journal of nutrition and dietetics (2025) 14 (2) 68-74

INTEGRATED THERAPY WITH GLP-1 RECEPTOR AGONISTS AND NUTRITIONAL INTERVENTIONS IN THE TREATMENT OF OBESITY IN PATIENTS WITH TYPE 2 DIABETES

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Summary

Obesity and type 2 diabetes represent a major public health challenge, and their strong interrelationship requires integrated therapeutic approaches. GLP-1 receptor agonists have, in recent years, taken a central role in the pharmacotherapy of diabetes and obesity due to their effects on appetite regulation, body weight reduction, and glycaemic control. At the same time, wellstructured medical nutrition therapy remains the foundation of non-pharmacological weightmanagement strategies. However, the combination of GLP-1 receptor agonists with individualized dietary interventions is still insufficiently explored in real-world clinical settings. The aim of this study was to investigate the effects of combined therapy with GLP-1 RA and nutritional interventions in patients with type 2 diabetes and obesity, and to compare the findings with those reported in the SUSTAIN and LEADER clinical trial programs**.** Parameters monitored over a six-month period included body weight, body mass index, waist circumference, HbA1c, blood pressure, lipid profile, renal parameters, and liver enzymes. The results show that combined therapy leads to statistically and clinically significant improvements in all major metabolic outcomes. Body weight decreased by −8.60 kg (−7.82%), BMI by −3.14 kg/m², waist circumference by −4.97 cm, and HbA1c by −1.99%. Additionally, moderate reductions in blood pressure, total cholesterol, and ALT levels were observed. The findings indicate that the combination of GLP-1 RA and well-guided nutritional therapy produces stronger effects than those reported in the SUSTAIN and LEADER programs. This study confirms that an integrated approach, combining pharmacological therapy with individualized nutritional support, represents an optimal model for managing obesity in patients with type 2 diabetes.

Keywords: GLP-1 RA, semaglutide, liraglutide, obesity, type 2 diabetes, medical nutrition therapy

Introduction

Obesity and type 2 diabetes mellitus (T2DM) represent two of the most prevalent and clinically significant metabolic disorders worldwide. Their close pathophysiological relationship is primarily driven by insulin resistance, systemic inflammation, and impaired regulation of energy balance. Obesity increases the risk of developing type 2 diabetes by three to five times, while the presence of diabetes further complicates weight reduction and the maintenance of achieved weight loss. Glucagon-like peptide-1 receptor agonists (GLP-1 RA) have in recent years become cornerstone therapies for the management of both diabetes and obesity. Their mechanisms of action include delayed gastric emptying, appetite suppression, enhanced insulin secretion, and reduced glucagon release. Clinical studies, including the SUSTAIN program, have demonstrated that GLP-1 RAs significantly reduce HbA1c and lead to moderate reductions in body weight in patients with T2DM. Despite these advances, dietary intervention remains a crucial component of obesity management. Caloric restriction, increased intake of dietary fiber, and reduction of refined carbohydrates have been shown to contribute to weight loss, reductions in abdominal obesity, and improved glycemic control. However, long-term patient adherence remains a major challenge.

Because GLP-1 RAs facilitate dietary adherence by reducing hunger and preference for energy-dense foods, it is hypothesized that combining pharmacotherapy with personalized nutrition may yield superior outcomes compared to either intervention alone. The aim of this study was to evaluate the metabolic effects of combined GLP-1 RA therapy and structured dietary intervention in patients with type 2 diabetes and obesity and to compare these findings with results from the SUSTAIN (Semaglutide Unabated Sustainability in Treatment of Type 2 Diabetes) and LEADER (Liraglutide Effect and Action in Diabetes: Evaluation of Cardiovascular Outcome Results) clinical programs.

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Mathreials and methods

Study design

The study was conducted at the Public Health Institution "Dom zdravlja Živinice," within the Consultative-Specialist Service, and was designed as a six-month prospective clinical investigation. This design enabled follow-up of changes in metabolic and anthropometric parameters within the same group of participants across predefined time intervals. The prospective approach ensured systematic and chronologically consistent data collection, reducing the risk of bias typical of retrospective studies. The obtained results were compared with findings from relevant international clinical trials—primarily SUSTAIN and LEADER—to assess the alignment of local outcomes with established evidence on the efficacy and safety of GLP-1 receptor agonists. Throughout the study, participants attended regular follow-up visits according to a predetermined schedule, which enhanced the consistency and reliability of the collected data.

Participants

The study included 30 patients with confirmed type 2 diabetes and obesity, with a body mass index (BMI) ≥ 35 kg/m². All participants were adults, clinically stable, and under regular supervision of a diabetologist. Inclusion criteria were: age ≥ 18 years; type 2 diabetes mellitus of at least one year in duration; stable antidiabetic therapy for at least three months prior to enrollment; BMI ≥ 35 kg/m², and ability to comply with the study protocol. Exclusion criteria were:acute or chronic liver or kidney disease; active malignancy; type 1 diabetes; pregnancy and breastfeeding.

Interventions

*Pharmacological intervention***.** All participants received treatment with GLP-1 receptor agonists—liraglutide or semaglutide—in accordance with current guidelines from the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD) for the management of T2DM and obesity, ensuring ethical justification and avoiding any experimental or off-label approaches. Liraglutide was administered subcutaneously once daily, while semaglutide was administered subcutaneously once weekly.
Dosing was individualized based on tolerance and therapeutic response. Throughout the study, side effects, adherence, and metabolic response were systematically monitored.

Nutritional intervention. In addition to pharmacotherapy, all participants followed a structured dietary plan consistent with modern nutritional recommendations for individuals with T2DM and obesity. The intervention included: a daily caloric deficit of 500–750 kcal; increased intake of dietary fiber (whole grains, vegetables, legumes); reduced intake of refined carbohydrates, saturated fats, and industrially processed foods.

Participants attended monthly sessions with a nutritionist to monitor adherence, support motivation, and adjust dietary plans to individual needs. The integration of pharmacological and nutritional therapy enabled a synergistic effect, consistent with current approaches to managing T2DM and obesity.

Statistical analysis

Descriptive statistics were used to present the baseline characteristics of the sample. To compare data across the three time points in baseline, after 3 months, and after 6 months a one-way repeated-measures analysis of variance (ANOVA) was applied, followed by Bonferroni post-hoc testing to identify statistically significant differences between intervals. A p-value < 0.05 was considered statistically significant.

Results

Table 1 presents the basic characteristics of the participants, including age and diabetes duration. The mean age of the study sample was 59.9 ± 6.5 years, while the average duration of diabetes was 8.73 ± 5.65 years. These data indicate a relatively homogeneous baseline sample structure, which is essential for valid interpretation of changes observed following the intervention (Sorli et al., 2017; Pratley et al., 2018). Sample homogeneity reduces the influence of potential confounding factors and allows more reliable conclusions regarding the effects of the treatment.

Table 1. Baseline characteristics of participants

Variable	Value
Number of participants	30
Age (years)	59.9 ± 6.5
Duration of T2DM (years)	8.73 ± 5.65

Table 2 shows the trajectory of body weight reduction throughout the study period. A clear progressive decrease in body weight was observed, with a statistically significant reduction already after three months of intervention (−5.04 kg; $p < 0.001$). This trend continued in the following period, resulting in a total mean weight loss of 8.60 kg after six months ($p < 0.001$). These findings indicate a stable and clinically meaningful improvement in weight control.

Table 2. Changes in body weight (BW)

Time period	BW (kg)	Change (kg)	p
Baseline	110.00	—	—
3 months	104.96	−5.04	<0.001
6 months	101.40	−8.60	<0.001

Table 3 illustrates the changes in BMI during the follow-up period. A statistically significant reduction in BMI was observed after three months (−1.78 kg/m²; $p < 0.001$), which further deepened by the sixth month, reaching a total reduction of −3.14 kg/m² ($p < 0.001$). These findings reflect a continuous and clinically relevant reduction in adipose tissue mass, consistent with the expected physiological effects of the intervention. The results confirm the effectiveness of the treatment in improving anthropometric parameters over time.

Table 3. Changes in Body Mass Index (BMI)

Time period	BMI (kg/m²)	Change (kg/m²)	P
Baseline	40.95	—	—
3 months	39.17	−1.78	<0.001
6 months	37.81	−3.14	<0.001

Changes in waist circumference presented in Table 4 show a significant decrease at both time points—after three and after six months (−3.04 / −4.97 cm), reflecting a reduction in visceral adipose tissue, one of the key predictors of cardiometabolic risk.

Table 4. Changes in waist circumference (WC)

Time period	WC (cm)	Change (cm)	P
Baseline	121.27	—	—
3 months	118.23	−3.04	<0.001
6 months	116.30	−4.97	<0.001

The reduction in HbA1c presented in Table 5 amounting to −1.99% after six months of therapy, represents a remarkably strong therapeutic response. A statistically significant decrease in HbA1c was observed as early as the three-month follow-up, with further improvement at six months.

Table 5. Changes in HbA1c

Time period	HbA1c (%)	Change (%)	P
Baseline	8.88	—	—
3 months	7.07	−1.81	<0.001
6 months	6.89	−1.99	<0.001

Table 6 shows the reduction in systolic and diastolic blood pressure after six months of therapy, reaching statistical significance ($p = 0.02$).

Table 6. Changes in blood pressure after 6 months of therapy

Parameter	Blood pressure (mmHg)	p
Systolic BP	−6.03 mmHg	0.02
Diastolic BP	−3.28 mmHg	0.02

Table 7 demonstrates a reduction in total cholesterol of 0.71 mmol/L, which is statistically significant ($p = 0.01$). Although triglycerides decreased by 0.54 mmol/L, this change did not reach statistical significance ($p = 0.17$).

Table 7. Changes in lipid profile

Parameter	Lipids (mmol/L)	p
Total cholesterol	−0.71 mmol/L	0.01
Triglycerides	−0.54 mmol/L	0.17

The reduction in liver enzymes ALT and AST is shown in Table 8. A significant reduction in ALT (−6.60 U/L) was observed ($p = 0.01$), while the decrease in AST was minimal and statistically non-significant ($p = 0.88$).

Table 8. Liver function parameters

Parameter	Status	P
ALT (Alanine aminotransferase)	−6.60 U/L	0.01
AST (Aspartate aminotransferase)	stable	0.88

Renal function parameters presented in Table 9 remained stable throughout the entire follow-up period. No statistically significant changes were observed in urea or creatinine levels ($p = 0.17$), indicating that the applied intervention had no adverse effects on kidney function. These results further support the safety profile of the treatment in relation to monitored renal biomarkers.

Table 9. Renal function parameters

Parameter	Status	P
Urea	stable	0.17
Creatinine	stable	0.17

Table 10 presents the results of repeated-measures ANOVA for three key anthropometric variables: body weight (BW), waist circumference (WC), and BMI at baseline, 3 months, and 6 months. Body weight showed a continuous and statistically significant decline, with a very strong effect size ($\eta^2 = 0.71$). Waist circumference also decreased progressively, reflecting reductions in abdominal adiposity, with an equally strong treatment effect ($\eta^2 = 0.71$). BMI demonstrated a consistent downward trend across all time points, accompanied by a large effect size ($\eta^2 = 0.67$), further confirming the intervention's effectiveness in improving body composition.

Table 10. Analysis of variance with repeated measurements of the variables body weight, waist circumference and body mass index

Variables	Time period	N	M	SD	Wilks lambda	F	p	Partial eta squared
BW	Baseline	30	110.00	20.43	—	—	—	—
	3 months	30	104.96	20.30	0.29	34.71	0.00	0.71
	6 months	30	101.40	20.18	—	—	—	—
WC	Baseline	30	121.17	12.61	—	—	—	—
	3 months	30	118.23	12.42	0.29	34.16	0.00	0.71
	6 months	30	116.30	12.14	—	—	—	—
BMI	Baseline	30	40.95	4.20	—	—	—	—
	3 months	30	39.17	4.22	0.33	28.04	0.00	0.67
	6 months	30	37.81	4.23	—	—	—	—

Highly statistically significant differences were observed across all three variables over time ($p < 0.001$), with Wilks' lambda values ranging from 0.29 to 0.33, indicating a strong multivariate effect of time—i.e., of the therapeutic intervention. The partial eta-squared coefficients ($\eta p^2 = 0.67–0.71$) indicate a very large effect size, meaning that GLP-1 agonists combined with dietary intervention explained more than 67% of the variance in BW, WC, and BMI.

Discussion

The results of this study show that the combination of GLP-1 receptor agonists and medical nutrition therapy leads to markedly better metabolic and anthropometric outcomes compared with standard therapy in patients with type 2 diabetes and obesity. To better understand the significance of these findings, they were systematically compared with the results of two of the most relevant groups of clinical trials: the SUSTAIN program, which evaluated semaglutide in various populations of patients with T2DM, and the LEADER trial, the key cardiovascular outcome trial assessing the efficacy and safety of liraglutide in individuals with type 2 diabetes.

Comparison with SUSTAIN 1–7 shows that the effects of our intervention on body weight reduction (−7.82%) and HbA1c improvement (−1.99%) are stronger than the typical values reported in those trials (3.7–6.9% weight loss; HbA1c reduction of 1.2 to 1.8%). Similarly, comparison with the LEADER trial indicates that other metabolic markers, such as blood pressure, abdominal obesity, and total cholesterol, also improved to a greater extent in our study. This difference most likely arises from combining pharmacotherapy with a structured dietary intervention, as well as from the fact that our participants were treated with more potent GLP-1 receptor agonists (semaglutide), whereas LEADER included liraglutide only.

Taken together, both sources of evidence confirm a consistent direction of effect of GLP-1 receptor agonists, while at the same time highlighting that an integrated therapeutic approach, such as the one applied in our study, can lead to faster and more pronounced improvements. Below, we provide a detailed overview of the key differences in outcomes between our study and the SUSTAIN and LEADER trials.

Our study demonstrated that the combination of GLP-1 RAs and medical nutrition therapy yields superior clinical effects compared with standard therapy. The results exceeded those of the SUSTAIN trials, in which weight loss ranged from 3.7 to 6.9%, whereas in our study it reached 7.82%.

The outcomes of our research surpass the typical effects of GLP-1 RAs reported in the SUSTAIN trials, where reductions in body weight were less pronounced (3.5–6.5 kg) in individuals with T2DM (Aroda et al., 2017; Sorli et al., 2017), suggesting synergy with the dietary intervention. The reduction in BMI (−3.14 kg/m²) indicates a statistically significant loss of adipose tissue. A similar but less pronounced effect of GLP-1 RAs on BMI was reported in SUSTAIN 2 and SUSTAIN 3 (Aroda et al., 2017; Ahmann et al., 2018), which further underscores the importance of integrated nutritional support.

The significant reduction in waist circumference at both time points in our study, after three and six months (−3.04 / −4.97 cm), reflects a decrease in visceral adipose tissue, one of the key predictors of cardiometabolic risk. This magnitude of change is comparable to the MRI findings from SUSTAIN 7, which showed a 15–20% reduction in visceral fat (Pratley et al., 2018).

The reduction in HbA1c of −1.99% after six months of therapy represents a remarkably strong therapeutic response. A statistically significant reduction in HbA1c in our study was achieved as early as three months, with a further decline observed at six months, exceeding the results of the SUSTAIN program (−1.2 to −1.8%), including SUSTAIN 6 (Marso et al., 2016). Possible explanations include higher baseline BMI, better adherence, and structured dietary support.

The decreases in systolic (−6.03 mmHg) and diastolic blood pressure (−3.28 mmHg) in patients treated with GLP-1 RAs in our study are in line with previous findings indicating that GLP-1 RAs exert beneficial effects on hemodynamic parameters, primarily via weight reduction and improved vascular function (Marso et al., 2016; Wilding et al., 2021).

The reduction in total cholesterol and triglycerides indicates a favourable effect of therapy on the lipid profile, although the decrease in triglycerides was numerically present but did not reach statistical significance. These results are consistent with findings from SUSTAIN 3 and SUSTAIN 7, which report modest but consistent improvements in lipid parameters (Aroda et al., 2017; Pratley et al., 2018).

The significant reduction in ALT values in our study (−6.60 U/L) suggests regression of steatohepatitis and improvement in liver functional status, which is consistent with earlier studies showing a hepatoprotective effect of GLP-1 RAs (Madsbad, 2016; Nauck & Meier, 2019). Stable AST values indicate the absence of hepatotoxicity.

The stability of urea and creatinine confirms the favourable renal safety profile of GLP-1 RA therapy. These findings are fully consistent with long-term safety data for GLP-1 RAs reported in SUSTAIN 6 and other cardiovascular outcome trials (Marso et al., 2016; Nauck & Meier, 2019). The beneficial effects on blood pressure, lipids, and liver enzymes confirm the systemic benefits of the therapy. Study limitations include the small sample size and non-randomized design, but the results strongly support the use of integrated treatment.

Table 11. Comparison of results from our study and the SUSTAIN clinical program

Outcome	Our study (liraglutide/semaglutide + diet, 6 months)	SUSTAIN 1–7 (semaglutide in T2DM)	Reference
Body weight (kg)	−8.60 (−7.82%)	−3.5 to −6.5	Sorli et al., 2017; Aroda et al., 2017
HbA1c (%)	−1.99	−1.2 to −1.8%	Marso et al., 2016; Davies et al., 2021
Waist circumference (cm)	−4.97	Reduction in visceral fat 15–20%	Pratley et al., 2018
Systolic blood pressure (mmHg)	−6.03	−2 to −5	Marso et al., 2016
Diastolic blood pressure (mmHg)	−3.28	−1 to −2	Marso et al., 2016
Total cholesterol (mmol/L)	−0.71	Moderate reduction	Aroda et al., 2017
ALT (U/L)	−6.60	−4 to −7 U/L	Aroda et al., 2017
Renal function	Stable	Stable	Nauck & Meier, 2019

The LEADER trial is a large, randomized, placebo-controlled cardiovascular outcome study (CVOT) in patients with type 2 diabetes and high cardiovascular risk, published in 2016. It demonstrated that liraglutide reduces the risk of cardiovascular events in patients with type 2 diabetes and increased cardiovascular risk.

Comparison of the results of our six-month intervention (combination of liraglutide/semaglutide and targeted diet) with the outcomes of the LEADER trial, in which liraglutide alone was evaluated in individuals with type 2 diabetes, indicates a consistent direction of effect but considerably more pronounced improvements in almost all parameters in our population (Marso et al., 2016).

The greatest difference is observed in body weight reduction, where our participants achieved an average loss of −8.60 kg (−7.82%), which clearly exceeds the results of LEADER (−2.3 to −3.0 kg). This difference is likely due to two factors: the inclusion of a more potent GLP-1 receptor agonist (semaglutide) and the structured dietary program, which was not present in LEADER. Similarly, the reduction in HbA1c in our study (−1.99%) is almost twice that reported in

LEADER, indicating a stronger glycaemic response with the integrated therapeutic approach.

Blood pressure parameters (systolic and diastolic) also show greater improvements in our study, which can be partly explained by the greater weight loss, improved metabolic status, and potential additional effects of semaglutide. The reduction in waist circumference (−4.97 cm) further confirms substantial visceral fat loss, whereas the LEADER trial reports only moderate, non-quantified reductions.

Regarding the lipid profile, our study shows a moderate reduction in total cholesterol (−0.71 mmol/L), which is more pronounced than the modest changes seen in LEADER (≈0.2–0.3 mmol/L). ALT results are comparable, with both studies confirming the hepatoprotective potential of GLP-1 receptor agonists. Finally, renal function remained stable in both studies, although LEADER additionally points to a slowing of the progression of microvascular damage during long-term follow-up (Marso et al., 2016).

Overall, our study demonstrates stronger metabolic and anthropometric effects compared with LEADER, underscoring the importance of combining GLP-1 receptor agonist pharmacotherapy with targeted dietary interventions, as well as the use of more potent, newer-generation agents.

Table 12. Comparison of results from our study and the LEADER trial

Outcome	Our study (liraglutide/semaglutide + diet, 6 months)	LEADER trial (liraglutide in T2DM)
Body weight (kg)	−8.60 (−7.82%)	−2.3 to −3.0 kg
HbA1c (%)	−1.99%	−1.0 to −1.3%
Waist circumference (cm)	−4.97 cm	Moderate reduction; not quantified
Systolic blood pressure (mmHg)	−6.03 mmHg	−2.3 to −3.0 mmHg
Diastolic blood pressure (mmHg)	−3.28 mmHg	−1.5 to −2.0 mmHg
Total cholesterol (mmol/L)	−0.71 mmol/L	Mild reduction (≈0.2–0.3 mmol/L)
ALT (U/L)	−6.60 U/L	Reduction of 5–7 U/L
Renal function	Stable	Stable / slowed progression of nephropathy

Conclusion

In the study, patients treated with GLP-1 receptor agonists achieved a total body weight reduction of 8.6 kg, or −7.82%, which is superior to results from the SUSTAIN trials, where semaglutide produced an average weight reduction between −3.7% and −6.9%, depending on dose and study population. Similar to the SUSTAIN program, the greatest weight loss occurred during the first three months, followed by continued steady reduction through month six.

The reduction in HbA1c observed in the study (−1.99%) was greater than that reported in the SUSTAIN trials (semaglutide: −1.2 to −1.8%) and the LEADER trial (liraglutide: −1.0 to −1.3%), further highlighting the importance of medical nutrition therapy and the benefits of an integrated treatment approach (pharmacotherapy + diet therapy). As in the SUSTAIN and LEADER programs, the largest improvement in HbA1c occurred within the first 12 weeks, followed by stabilization of glycemic control. The results of the study demonstrated a reduction in waist circumference of −4.97 cm and a total decrease in BMI of −3.14 kg/m². Comparable but smaller changes in anthropometric parameters have been reported in the SUSTAIN program, where GLP-1 receptor agonists are particularly effective in reducing abdominal adiposity—a key determinant of cardiometabolic risk. Alsom the study documented a statistically significant decrease in both systolic and diastolic blood pressure, with a more pronounced effect after six months. These findings are consistent with the LEADER trial. The study results demonstrated reductions in total cholesterol and ALT values, which align with findings from the SUSTAIN studies. Urea and creatinine levels showed no significant changes, consistent with the LEADER trial, in which liraglutide exhibited a neutral or mildly protective effect on renal function.

When comparing the study outcomes with the large-scale global clinical trials SUSTAIN and LEADER, it may be concluded that the use of GLP-1 receptor agonists combined with targeted nutritional intervention represents an effective, safe, and metabolically superior therapeutic strategy for patients with type 2 diabetes and obesity.

References

Ahmann, A. J., Capehorn, M., Charpentier, G., Dotta, F., Henkel, E., Lingvay, I., Maas, S. E., McGuire, M. T., Nauck, M., Pratley, R. E., Thomsen, M., Meier, J. J. (2018): Semaglutide once weekly versus exenatide extended release in SUSTAIN 3, *Diabetes Care* 41(2), 258–266.

Aroda, V. R., Ahmann, A., Cariou, B., Chow, F., Duprat, L., Franek, E., Ørsted, D. D., Pieber, T. R., Meier, J. J. (2017): Comparative efficacy of semaglutide versus sitagliptin as add-on therapy to metformin and/or a sulfonylurea in SUSTAIN 2, *Diabetes Care* 40(7), 123–131.

Bray, G. A., Kim, K. K., Wilding, J. P. H. (2018): Obesity: A chronic relapsing progressive disease process, *Clinical Obesity* 8(5), 304–310.

Coleman, N. J., Muthu, V., Chadalavada, K., Wilson, K., Ramanathan, R. (2022): Obesity and diabetes: Mechanisms and clinical implications, *Diabetes Research and Clinical Practice* 187, 109–119.

Davies, M. J., Aroda, V. R., Collins, B. S., Gabbay, R. A., Green, J., Marso, S. P., Mathieu, C. (2021): Management of hyperglycemia in type 2 diabetes, *Diabetologia* 64, 1925–1966.

Hall, K. D., Kahan, S. (2018): Maintenance of lost weight and long-term management of obesity, *Medical Clinics of North America* 102(1), 183–197.

Madsbad, S. (2016): Clinical implications of treatment with GLP-1 receptor agonists, *Diabetes* 65, 1335–1345.

Marso, S. P., Bain, S. C., Consoli, A., Eliaschewitz, F. G., Jódar, E., Leiter, L. A., Lingvay, I., Rosenstock, J., Seufert, J., Warren, M. L., Woo, V., Hansen, O., Holst, A. G., Pettersson, J., Vilsbøll, T. (2016): Semaglutide and cardiovascular outcomes in patients with type 2 diabetes (SUSTAIN 6), *New England Journal of Medicine* 375(19), 1834–1844.

Marso, S. P., Daniels, G. H., Brown-Frandsen, K., Kristensen, P., Mann, J. F. E., Nauck, M. A., Nissen, S. E., Pocock, S., Poulter, N. R., Riddle, M. C., Steinberg, W. M., Stockner, M., Zinman, B., Bergenstal, R. M., Buse, J. B. (2016): Liraglutide and cardiovascular outcomes in type 2 diabetes (LEADER trial), *New England Journal of Medicine* 375(4), 311–322.

Müller, T. D., Finan, B., Tschöp, M. H. (2017): The role of GLP-1 in energy homeostasis, *Nature Reviews Endocrinology* 13, 703–716.

Nauck, M. A., Meier, J. J. (2019): Safety of GLP-1 receptor agonists, *Nature Reviews Gastroenterology & Hepatology* 16(9), 508–523.

Pratley, R. E., Aroda, V. R., Lingvay, I., Lüdemann, J., Andreassen, C. S., Navarria, A., Vilsbøll, T., Meier, J. J. (2018): Semaglutide versus dulaglutide once weekly in type 2 diabetes (SUSTAIN 7), *Lancet Diabetes & Endocrinology* 6(4), 275–286.

Rodriguez, A., Manley, S., Nadeau, K. J., Oliver, N., Pratley, R. E., Sodemann, U., Thomsen, M., Capehorn, M. (2018): Once-weekly semaglutide added to basal insulin therapy (SUSTAIN 5), *Diabetes, Obesity & Metabolism* 20(11), 2755–2764.

Rubino, D., Abrahamsson, N., Davies, M. J., Hesse, D., Greenway, F., Jensen, C. (2022): Combining GLP-1 receptor agonists with dietary interventions, *Obesity Reviews* 23(4), e13457.

Sorli, C., Harashima, S.-I., Tsoukas, G. M., Unger, J., Karsbøl, J. D., Hansen, T., & Bain, S. C. (2017): Efficacy and safety of once-weekly semaglutide monotherapy versus placebo in patients with type 2 diabetes (SUSTAIN 1), *Diabetes Care* 40(7), 1177–1185.

Wilding, J. P. H., Batterham, R. L., Calanna, S., Davies, M., Van Gaal, L., Lingvay, I., McGowan, B., Rosenstock, J., Tran, M. T. D., Wadden, T. (2021): Once-weekly semaglutide in adults with overweight or obesity, *New England Journal of Medicine* 384, 989–1002.

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THE ROLE OF CITICOLINE IN OCULAR NEURODEGENERATIVE DISEASES

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review paper

Summary

Ocular neurodegenerative diseases such as glaucoma, diabetic retinopathy and age-related macular degeneration are the most common retinal diseases causing visual impairment and blindness in the working age elderly population in developed countries. Conventional therapy is either used in an advanced stage of the disease or is not sufficient on its own. Therefore, new treatment modalities are sought, especially in the earlier stages of the disease, in order to prevent disease progression before visual acuity is impaired, and patient's quality of life is altered. Neuroprotection is increasingly popular term used not only to describe the pathology of the central nervous system, but also eye diseases. One of the proposed neuroprotectors of the eye is citicoline. This review provides comprehensive data on the use of citicoline in the most common ocular neurodegenerative diseases.

Keywords: neurodegeneration; neuroprotection; citicoline; eye; brain

Introduction

Human eye is composed of three concentric and distinct layers of tissue: the sclera, the uvea, and the retina. It is a laminar tissue containing neurons and glial cells that plays the crucial role of phototransduction, the process of converting light energy into encoded neural signals delivered to the brain (Ptito et al., 2021). The eye is rightly considered as an extension of the brain because both organs are composed of nerve cells and are derived from the neural tube. The eye provides a unique window to the brain due to its special connection by the retina, a photosensitive nerve tissue that covers the inner surface of the eye, which is properly considered as a part of the brain. Light passes through the cornea, pupil, lens, and vitreous humor and reaches the retina, generating visual stimuli from the outside world, which are converted into electrical impulses and transported through the optic nerve to the brain. The brain interprets them to create a meaningful image. Therefore, the retina is a functional unit of the central nervous system that converts light signals into nerve impulses, and is physically connected to the brain via the axons of the optic nerve.

Chronic progressive neurodegeneration of the retina, which can occur in older adults, can lead to various eye disorders such as glaucoma, age-related macular degeneration (AMD), and diabetic retinopathy (DR). In the elderly population mainly, but also among younger people, this type of eye pathology is the cause of irreversible vision damage or loss (Figure 1).

Typical neurodegenerative diseases of the central nervous system (CNS) are a group of diseases with common characteristics and an etiology that is not fully understood; some risk factors have been identified, but they are not sufficient to explain all observed cases. Furthermore, several studies have shown that ocular disorders also represent features of neurodegenerative diseases, and on the other hand, CNS diseases, such as Alzheimer's disease and Parkinson's disease, show specific changes in the eye (Marchesi et al., 2021).

The most well known, widespread, and common diseases among the elderly population are Alzheimer's disease and Parkinson's disease, which affect more than 30 million and 5 million people worldwide, respectively. For some time, science has been studying whether retinal neurodegeneration can be a predictive factor for Alzheimer's disease and Parkinson's disease and found the changes in the microvasculature of the retina causing retinal thinning (Marchesi et al., 2021) or retinal nerve fiber layer thinning (Guidoboni et al., 2020). Of particular interest is that Alzheimer's disease and age-related macular degeneration share the same biomarkers (Marchesi et al., 2021).

Other neurodegenerative diseases of the CNS are also associated with eye damage, such as multiple sclerosis and amyotrophic lateral sclerosis (Soldatov et al., 2021; Marchesi et al., 2021). Evidence suggests that the cause of DR is not only microvascular damage and that retinal neurodegeneration is a critical component of its development (Mavija, 2020).

The eye - especially its parts such as the retina and the optic nerve represent an extension of the CNS. These

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parts of CNS are the only one that can be seen non-invasively (by fundus examination). Markers of retinal neurodegeneration are associated with markers of brain atrophy, therefore certain diagnostic methods such as optical coherence tomography (OCT) of the retina can also provide information about neurodegeneration in the brain (Mutlu et al., 2017).
The incidence of neurological diseases increases dramatically with age, but as life expectancy increases, the demand for high quality of life in older age becomes a priority for today's health care. Early diagnosis and optimal monitoring are critical for better disease management and to delay progression and disability. It is obvious that neurological and ophthalmological neurodegenerative diseases share underlying biology and pathophysiology which means that they share certain diagnostic biomarkers as well as therapies, especially in the form of neuroprotection.

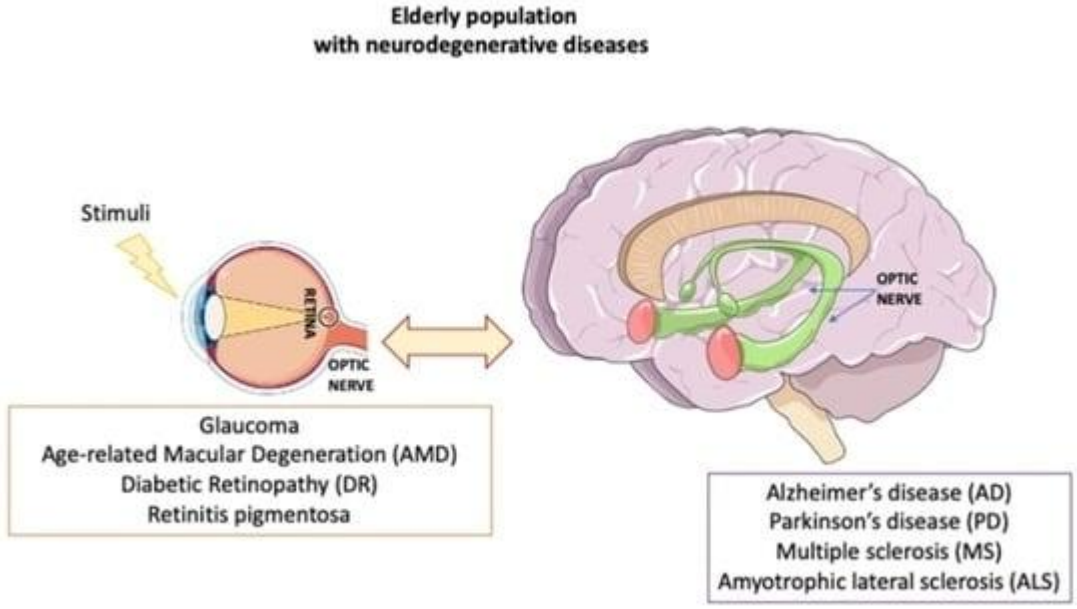

Figure 1. The most common ocular and brain neuodegenerative diseases (Marchesi et al., 2021)

Citicoline: chemical properties and health benefits

Citicoline is the international name for cytidine-diphosphocholine (CDP-Cho).
It is not typically found in food, but is formed in the body when choline combines with cytidine.
Citicoline is a precursor of both phosphatidylcholine, contributing to the synthesis of structural phospholipids in cell plasma membranes, and acetylcholine, an important neurotransmitter in cell metabolism (Garcia-Lopez et al., 2023).
While choline is an essential nutrient, the citicoline level may increase when taking food rich with choline (Table 1). Total choline content is higher in foods of animal origin, compared to foods of vegetable origin (Wiedeman et al., 2018). Upon oral intake, citicoline is thought to be rapidly absorbed and then hydrolyzed to choline and cytidine in the intestinal wall and liver: therefore, besides providing the metabolic precursors of phospholipids, enters synthetic pathways of nucleic acids, proteins, and acetylcholine (Weiss, 1995).

Citicoline can be used in food supplements aimed at a target population of middle-aged to elderly adults at a maximum level of 500 mg/day, and in dietary foods for special medical purposes with a maximum dose of 250 mg per serving and with a maximum daily consumption level of 1000 mg from these types of foods (Synoradzki and Grieb, 2019).
Whereas there is no nutrition or health claim authorized for cytidine either, there are three such claims authorized for choline. The first two state that choline contributes to normal lipid metabolism and to the maintenance of normal liver function and the third claim, stating that choline contributes to normal homocysteine metabolism (Synoradzki and Grieb, 2019). It is extensively researched for its other health benefits in neurology, ophthalmology and psychiatry. Citicoline is widely available as a dietary supplement in a form of tablets, an injection, oral formulation and topical solution for ocular use (Jasielski et al., 2020; Garcia-Lopez et al., 2023). In neurology, citicoline is know as a "procognitive" form of choline used in the preventin of dementia progression, improve memory

and cognitive function, reduce neuropathic pain and accelerated nerve regeneration, and improves prognosis after stroke (Jasielski et al., 2020; Synoradzki and Grieb, 2019; Bonvici et al., 2023; Turana et al., 2021).

Table 1. Food sources of choline (modified from Wiedeman et al., 2018)

Animal source	Plant source
Beef	Nuts (almonds)
Egg	Broccoli
Salmon	Beans
Pork	Red potato
Chicken	White rice
Milk	

The role of citicoline in ocular neurodegenerative diseases

The main ocular neurodegenerative disease include glaucoma, AMD, diabetic retinopathy, anterior ishemic optic neuropathy **(**NAION) and Retinis pigmentosa.

Although conventional medicine has seen substantial progress in recent years, there is a growing interest in nutraceuticals, bioactive compounds derived from natural sources such as plants, fruits, and cereals, due to their potential therapeutic applications. As it mentioned before the use of citicoline as a supplement is conducted in neurology, ophthalmology and psychiatry.

Citicoline was widely studied in systemic neurodegenerative diseases, like Alzheimer's disease, Parkinson's disease, and brain ischemia. The rationale for the use of citicoline in the aforementioned conditions based on its multifactorial mechanism of action (Oddone et al., 2021).

Glaucoma

Glaucoma is a degenerative neuropathy affecting the retinal ganglion cells. Currently it is the second leading cause of blindness worldwide and the third leading cause of visual impairment. The degeneration of these cells causes progressive optic atrophy that can lead to a complete loss of vision (Starvaggi et al., 2025). The major risk for glaucoma is increased intraocular pressure (IOP). Although the main therapy is conventional, such as the use of topical medicine in the form of drops and/or laser therapy, adjuvant therapy is also used in order to achieve better neuroprotection. The mainstay of current glaucoma therapy is limited to lowering IOP; however, controlling IOP in certain patients can be futile in slowing disease progression. The understanding of potential biomolecular processes that occur in glaucomatous degeneration allows for the development of glaucoma treatments that modulate the death of retinal ganglion cells and neuroprotection is currently the most important (Kuo and Liu, 2022).

Citicoline has been used to preserve the optic nerve since 1989 when it was used as an intramuscular injection. Today, citicoline is used in the form of drops and in the form of an oral solution in patients with eye problems and its important place in preserving the optic nerve, especially when it comes to the patient's field of vision (Giraldi et al., 1989).

Experimental studies have shown that citicoline may increase the synthesis of phospholipids in the CNS and has a neuromodulator effect, potentially leading to a protective effect on retinal ganglion cells, but also a multicenter study on oral citicoline supplementation in patients with progressive glaucomatous visual field loss found a reduction in the mean rate of visual field progression from −1 dB/year to −0.15 (±0.3) dB/year over a 2-year period (Starvaggi et al., 2025). A randomized placebo clinical trial shown that additional treatment with citicolineeyedrops to IOP-lowering treatment might reduce disease progression in patients with progressing glaucoma despite intraocular pressure ≤18 mm Hgfrom 1.86 μm of RNFL in 3 years in citicoline group, versus 2.99 μm in the placebo group (Rosseti et al., 2020).

Anterior ishemic optic neuropathy (AION)

AION is the most common type of acute optic neuropathy in subjects that were aged more than 50 years, due to a deficit of blood supply to the optic nerve from the posterior ciliary arteries. It represents an ophthalmological emergency, which is characterized by sudden and severe visual loss, visual field deficit, peripapillary hemorrhages, and optic nerve head swelling. AION can be non-arteritic (NAION) or arteritic (AAION), with a different prognosis, commonly being NAION, a self-limiting process accompanied by a partial resolution of the visual loss, and leading AAION to a more detrimental impairment of vision up to blindness (Odonne et al., 2021).

Unilateral optic disc edema and abrupt, painless vision loss are its defining features. It is commonly assumed that NAION is caused by an ischemic infarction of the optic nerve head, and, although the exact pathogenesis is still unknown. NAION occurs generally in patients older than 50 years who have small optic discs and vasculopathy risk factors. Even though numerous treatment options have been

proposed, no available effective medical or surgical therapy or prophylactic measure currently exists (Salvetat et al., 2023).

Visual loss in NAION is typically acute and stable, although a further worsening in the first days after presentation is not uncommon; however, the clinical course of NAION stabilizes within a few weeks, at most in 2–3 months, in the majority of patients. But it has a bad prognosis: it results in permanent vision loss, with 40–50% of patients having significant visual impairment or being legally blind in the affected eye (Salvetat et al., 2023).

Regarding the pharmacological management, it is generally accepted that systemic steroid therapy during early stages of NAION has a significant beneficial effect for visual outcome (Odonne et al., 2021; Hayrey et al.,2001; Al-Zubidi et al., 2014). Considering that retinal ganglion cells death is the final consequence of the ischemia in NAION eyes, neuroprotection strategies have been suggested as a potential treatment (Salvetat et al., 2023). Neuroprotective treatments have been reported to counteract secondary neurodegeneration of retinal ganglion cells and their axons used in appropriate interval and the rationale of neuroprotection in NAION resides in the fact that not 100% of the axons of the optic nerve die during an ischemic process, but only a portion of them is damaged. The surviving axons belonging to not damaged retinal ganglion cells, which are responsible for the residual visual acuity and visual field (Odonne et al., 2021).

Parisi et al. (2019) concluded improvement in visual acuity, visual field, VEP and retinal nerve fiber layer thickness after administration of citicoline 500 mg/day in the form of oral solution for six months in NAION patients.

Diabetic retinopathy (DR)

Diabetic retinopathy is the leading cause of new cases of blindness in patients with diabetes mellitus. In 2020, more than 103 million individuals with diabetes mellitus worldwide were affected by diabetic retinopathy, and estimates suggest this number will increase to 160 million by 2045 (Chong et al., 2024). DR represents one of the most important complications of diabetes and preventable causes of visual impairment among people of working age and industrialized countries (Oddone et al., 2021).

Due to constant and long-term elevated blood sugar levels, damage occurs to the small blood vessels of the retina, at the level of precapillary arterioles, capillaries and postcapillary venules. Changes in the fundus occur due to microvascular occlusion - the closure of these blood vessels, which results in ischemia (lack of oxygen) in the retinal tissue, and are manifested by capillary drop-out and neovascularization (new "unhealthy" blood vessels). These changes in the fundus progress further with the duration of the disease, but also by other factors in and outside the eye that contribute to the development of the disease (Mavija, 2020). In diabetes, vision loss also occurs due to leakage (leakage) from small blood vessels, which is manifested in the fundus as edema (swelling) of the macula. Therefore, diabetic macular edema occurs due to the accumulation of fluid within the layers of the retina in the center of macula lutea (fovea) and/or in its vicinity. It can occur as part of diabetic retinopathy or as an independent disease of the retina (Fung et al., 2022).

As mentioned above, diabetic retinopathy occurs due to microvascular damage to retinal capillaries. However, today there is abundant evidence that retinal (retinal) nerve dysfunction can occur long before the appearance of changes in the small blood vessels. Therefore, early diabetic retinopathy is considered not only as a vascular disease, but also as a neurovascular degenerative disease (Mavija, 2020).

Current treatment focus on the late stages of diabetic retinopathy, when vision is already significantly affected. Therefore, prevention and timely detection of early changes is necessary in order to prevent and/or delay the onset of an advanced stage of the disease in which the patient has already significantly lost vision.

When serious complications of diabetes have already occurred in the eye, treatment is based on: retinal laser photocoagulation, micropulse laser treatment (for diabetic macular edema), intravitreal drug administration in the operating room called antiVEGF (factors that prevent the growth of new blood vessels), and finally, vitrectomy.

Despite treatment, the fact remains that some patients continue to lose vision or do not see any improvement in vision after the resolution of macular edema due to retinal atrophy and/or ischemia (Simó et al., 2018).

Therefore, new and more effective preventive and interventional strategies are needed based on a better understanding of the pathogenesis of the early stages of the disease.

There are growing evidences clearly showing that neurodegeneration is an early event in the development of diabetic retinopathy that could be associated with the development of microvascular changes. Neurodegeneration has been shown to be present in the retina of diabetic patients even in the absence of clinically visible microvascular abnormalities. Therefore, neurodegeneration occurs

before microvascular disease, namely diabetic retinopathy (Simó et al., 2018; Khalaf et al., 2024).
Topical application of citicoline in the form of drops in combination with vitamin B12 has shown a protective effect on microvascular damage in diabetic retinopathy, as well as on the morphology of the retinal layers with stability of retinal function (Paravano et al., 2020). Tomić et al. (2025) shown a clinically positive effect of citicoline eye drops on hard exudates in DR.
Neuroprotection is considered a new treatment modality that can preserve retinal tissue, which further improves the outcome of treatment, especially in patients who are very "challenging" and incurable.

Age-related macular degeneration (AMD)

Age-related macular degeneration (AMD) is a disease of the macular area and is one of the most common causes of irreversible vision loss in people over 50 years of age. The main risk factor for the development of AMD is age over 50 years, followed by a positive family history, smoking, previous cataract surgery, atherosclerosis, obesity, arterial hypertension and cardiovascular disease, insufficient intake of vitamins A, C and E, omega 3 fatty acids, zinc and lutein in the diet and genetic factors (Čeklić and Mavija, 2015).
AMD is a multifactorial disease, in which the main factors for the development of the disease are changes in the structure and metabolism of a retina layer called the retinal pigment epithelium (RPE). The changes begin around the fourth decade of life and are more pronounced with increasing age (Hodžić et al., 2021).
Besides classic categorization on dry and wet macular degeneration, there are more detailed classifications. The classification of dry form of AMD relates to number and size of drusen and the changes in RPE. There is *early stage* with soft drusen, 64-124 μm in size and light pigment changes on the posterior pole, and *intermediate stage* with middle-sized drusen resulting in confluence and big drusen (> 125 μm) accumulated below retina. Late stage of AMD has two subtypes: *wet, exudative or neovascular* macular degeneration and *geographic atrophy* or dry degeneration (Hodžić et al., 2021). According to the literature, a dry form of AMD present in 10 - 20 % of patients progresses to wet form, where 40% of patients develop wet form of AMD on both eyes (Andrijević Derk, 2015).
If the wet form has developed (the late stage of the disease with neovascularization) the treatment choice is antiVEGF therapy, which inhibits vascular endothelial growth factor (VEGF) which is administered intravitreally. For patients with geographic atrophy (late-stage dry degeneration), an adequate therapy has not yet been found, and irreversible vision loss in AMD is primarily attributed to retinal neurodegeneration and choroidal neovascularization that occur in the later stages of the disease (Lin et al., 2022).
Neuroprotection has emerged as a strategy to delay photoreceptor (RPE cell) death and preserve vision. One of advantages of neuroprotection is that it have the ability to slow RPE degeneration regardless of the underlying cause and may even generalize to other neurodegenerative retinal diseases. Citicoline has been shown to attenuate photoreceptor death (apoptosis) and as such represents a new potential modality for preserving vision in individuals with AMD (Lin et al., 2022; Nashine and Kenney, 2020).

Conclusion

Citicoline is an essential compound for the proper functioning of the brain in general and the visual system in particular. Although its potential in delaying and onset of certain retinal diseases has only recently been discovered, citicoline has already found its place asn an adjuvant therapy in glaucoma and diabetic retinopathy among clinical ophthalmologists.

References

Al-Zubidi, N., Zhang, J., Spitze, A., Lee, A. G. (2014): Systemic corticosteroids in nonarteritic ischemic optic neuropathy, *Indian journal of ophthalmology* 62(10), 1022–1024.

Andrijević, Derk, B. (2015): Morfološke i angiografske promjene periferne mreznice u bolesnika sa senilnom makularnom degeneracijom (disertacija). Medicinski fakultet. Sveučilište u Zagrebu. Zagreb.

Bonvicini, M., Travaglini, S., Lelli, D., Antonelli Incalzi, R., Pedone, C. (2023): Is Citicoline Effective in Preventing and Slowing Down Dementia?-A Systematic Review and a Meta-Analysis, *Nutrients* 15(2), 386.

Čeklić, L., Mavija, M. (2015): Senilnadegeneracija makule. Praktični vodiči savremeni terapijski protokoli u prevenciji slabovidosti uzrokovane senilnom degeneracijom makule. Udruzenje Oftalmologa Republike Srpske.

Chong, D. D., Das, N., Singh, R. P. (2024): Diabetic retinopathy: Screening, prevention, and treatment, *Cleveland Clinic journal of medicine* 91(8), 503–510.

Fragiotta, S., Pinazo-Durán, M. D., Scuderi, G. (2022): Understanding Neurodegeneration from a Clinical and Therapeutic Perspective in Early Diabetic Retinopathy, *Nutrients* 14(4), 792.

Fung, T. H., Patel, B., Wilmot, E. G., Amoaku, W. M. (2022): Diabetic retinopathy for the non-ophthalmologist, *Clinical medicine (London, England)* 22(2), 112–116.

García-López, C., García-López, V., Matamoros, J. A., Fernández-Albarral, J. A., Salobrar-García, E., de Hoz, R., López-Cuenca, I., Sánchez-Puebla, L., Ramírez, J. M., Ramírez, A. I., Salazar, J. J. (2023): The Role of Citicoline and Coenzyme Q10 in Retinal Pathology, *International journal of molecular sciences* 24(6), 5072.

Guidoboni, G., Sacco, R., Szopos, M., Sala, L., VerticchioVercellin, A. C., Siesky, B., Harris, A. (2020): Neurodegenerative Disorders of the Eye and of the Brain: A Perspective on Their Fluid-Dynamical Connections and the Potential of Mechanism-Driven Modeling, *Frontiers in neuroscience* 14, 566428.

Hayreh, S. S., Podhajsky, P. A., Zimmerman, B. (2001): Ipsilateral recurrence of nonarteritic anterior ischemic optic neuropathy, *American journal of ophthalmology* 132(5), 734–742.

Hodžić, N., Kenjerić, D., Hodžić, Z., Nadarević-Vodenčarević, A. (2021): AGE-RELATED MACULAR DEGENERATION (AMD), SUPPLEMENTS AND NUTRITION - WHAT DOES THE EVIDENCE TELL US?, *Hrana u zdravlju i bolesti* 10(1), 14-23.

Jasielski, P., Pięđel, F., Piwek, M., Rocka, A., Petit, V., Rejdak, K. (2020): Application of Citicoline in Neurological Disorders: A Systematic Review, *Nutrients* 12(10), 3113.

Khalaf, H.S., Kim, L.A., Lim, J.I. (2024). Neurodegeneration in Diabetic Retinopathy. *American Academy of Ophthalmology*.

Kuo, C. Y., Liu, C. J. (2022): Neuroprotection in Glaucoma: Basic Aspects and Clinical Relevance, *Journal of personalized medicine* 12(11), 1884.

Lin, J. B., Murakami, Y., Miller, J. W., Vavvas, D. G. (2022): Neuroprotection for Age-Related Macular Degeneration, *Ophthalmology science* 2(4), 100192.

Marchesi, N., Fahmideh, F., Boschi, F., Pascale, A., Barbieri, A. (2021): Ocular Neurodegenerative Diseases: Interconnection between Retina and Cortical Areas, *Cells* 10(9), 2394.

Mavija M. (2020): Dijabetički makularni edem. Univerzitet u Banjoj Luci. Medicinski fakultet Banja Luka.

Nashine, S., Kenney, M. C. (2020): Role of Citicoline in an *in vitro* AMD model, *Aging* 12(10), 9031–9040.

Oddone, F., Rossetti, L., Parravano, M., Sbardella, D., Coletta, M., Ziccardi, L., Roberti, G., Carnevale, C., Romano, D., Manni, G., Parisi, V. (2021): Citicoline in Ophthalmological Neurodegenerative Disease: A Comprehensive Review, *Pharmaceuticals (Basel, Switzerland)* 14(3), 281.

Parisi, V., Barbano, L., Di Renzo, A., Coppola, G., Ziccardi, L. (2019): Neuroenhancement and neuroprotection by oral solution citicoline in non-arteritic ischemic optic neuropathy as a model of neurodegeneration: A randomized pilot study, *PloS one* 14(7), e0220435.

Parravano, M., Scarinci, F., Parisi, V., Giorno, P., Giannini, D., Oddone, F., Varano, M. (2020): Citicoline and Vitamin B_{12} Eye Drops in Type 1 Diabetes: Results of a 3-year Pilot Study Evaluating Morpho-Functional Retinal Changes, *Advances in therapy* 37(4), 1646–1663.

Pecori Giraldi, J., Virno, M., Covelli, G., Grechi, G., De Gregorio, F. (1989): Therapeutic value of citicoline in the treatment of glaucoma (computerized and automated perimetric investigation), *International ophthalmology* 13(1-2), 109–112.

Ptito, M., Bleau, M., Bouskila, J. (2021): The Retina: A Window into the Brain, *Cells* 10(12), 3269.

Rossetti, L., Iester, M., Tranchina, L., Ottobelli, L., Coco, G., Calcatelli, E., Ancona, C., Cirafici, P., Manni, G. (2020): Can Treatment With CiticolineEyedrops Reduce Progression in Glaucoma? The Results of a Randomized Placebo-controlled Clinical Trial, *Journal of glaucoma*, 29(7) 513–520.

Salvetat, M. L., Pellegrini, F., Spadea, L., Salati, C., Zeppieri, M. (2023): Non-Arteritic Anterior Ischemic Optic Neuropathy (NA-AION): A Comprehensive Overview, *Vision (Basel, Switzerland)* 7(4), 72.

Simó, R., Stitt, A. W., Gardner, T. W. (2018): Neurodegeneration in diabetic retinopathy: does it really matter?, *Diabetologia 61*(9), 1902–1912.

Synoradzki, K., Grieb, P. (2019): Citicoline: A Superior Form of Choline?, *Nutrients* 11(7), 1569.

Soldatov, V. O., Kukharsky, M. S., Belykh, A. E., Sobolev, A. M., Deykin, A. V. (2021): Retinal Damage in Amyotrophic Lateral Sclerosis: Underlying Mechanisms, *Eye and brain* 13, 131–146.

Tomić, M., Babić, T., Bulum, T., Ljubić, S., Jukić, T. (2025): Citicoline May Effectively Reduce Hard Exudates in Diabetic Retinopathy, *Biomedicines* 13(10):2358.

Turana, Y., Nathaniel, M., Shen, R., Ali, S., Aparasu, R. R. (2021): Citicoline and COVID-19-Related Cognitive and Other Neurologic Complications, *Brain sciences* 12(1), 59.

Weiss, G.B. (1995). Metabolism and actions of CDP-choline as an endogenous compound and administered exogenously as citicoline. *Life Sci* 56, 637–660.

Wiedeman, A. M., Barr, S. I., Green, T. J., Xu, Z., Innis, S. M., Kitts, D. D. (2018). Dietary Choline Intake: Current State of Knowledge Across the Life Cycle. *Nutrients* 10(10), 1513.

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THE EFFECT OF VITAMIN B_{12} DEFICIENCY ON NEUROLOGICAL AND COGNITIVE HEALTH OF THE ELDERLY: A REVIEW

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review paper

Summary

Vitamin B_{12} (cobalamin) is an essential nutrient necessary for DNA synthesis, fatty acid metabolism, and proper function of the nervous system. In the diet, it comes almost exclusively from foods of animal origin, while plant sources contain negligible amount or inactive forms of B_{12}. Elderly people are especially susceptible to the development of deficits due to reduced absorption, frequent chronic diseases, and use of medications that interfere with cobalamin metabolism. Subclinical deficit occurs in 15–40% of the elderly and often remains unrecognized, although it can gradually progress to neurological impairment. Absorption of vitamin B_{12} involves complex mechanisms that depend on the function of the stomach, pancreas, and distal ileum. Malabsorption occurs in atrophic gastritis, pernicious anemia, gastrointestinal diseases, and post-surgically. Bioavailability depends on the food source; it is the highest in meat and fish, while it is significantly lower in eggs and milk. Deficiency diagnosis is complex because neurological manifestations can occur even without the presence of anemia. Evaluation includes measurement of serum vitamin B_{12}, holotranscobalamin, homocysteine, and methylmalonic acid. Modern methods, such as the CobaSorb test, enable a more accurate assessment of absorption. Vitamin B_{12} deficiency can cause peripheral neuropathies, gait disorders, cognitive decline, psychiatric symptoms, and myelopathy, while excess often indicates more serious pathological processes such as liver, kidney, or malignancy. Timely recognition and adequate compensation are crucial in preventing permanent neurological damage, particularly among the elderly population.

Keywords: vitamin B12; food; deficiency; neurologic manifestations; elderly

Introduction

Vitamin B_{12} (cobalamin) is the most complex water-soluble vitamin of group B, necessary for DNA synthesis, nutrient metabolism, and normal functioning of the central and peripheral nervous system (Bundalo and Srkalović Imširagić, 2013; EFSA, 2015). In the diet, it is almost entirely found in foods of animal origin, since it is exclusively synthesized by certain microorganisms (some bacteria and archaea) in animal's gut (Watanabe and Bito, 2018). Therefore; the richest sources include liver and other offal, while plant foods contain negligible amounts of B_{12}, except for certain algae and fortified products like breakfast cereals or plant milks (Herbert, 1988; Carmel, 2011 b; Karakaš and Antonić, 2017; Nohr et al., 2016). The recommended daily intake is approximately 3 µg for adults, but the needs of older people are often increased due to reduced absorption and dietary restrictions (Miškulin et al., 2014; Wong, 2015; Vincenti et al., 2021). In addition to biological changes, the risk of deficiency is further increased by specific dietary patterns and socioeconomic factors (Porter et al., 2016). Subclinical vitamin B_{12} deficiency is particularly common, occurring in 15–40% of elderly, often without anemia, but with the possible gradual development of neurological damage (Clarke et al., 2007; Moore et al., 2012; Leishear et al., 2012). The use of more sensitive diagnostic methods, such as the determination of homocysteine and methylmalonic acid, enables earlier and more reliable recognition of this condition (Mathew et al., 2024).

This narrative review aims to synthesize current knowledge on the neurological manifestations of deficiency, outline the main diagnostic challenges, and highlight potential implications for clinical practice and public health.

Absorption and metabolism

Knowledge of the mechanisms of vitamin B_{12} absorption, transport, and metabolismis crucial for timely recognition of its deficiency, especially in at-risk populations. Maintaining an adequate nutritional status—by diet or supplementation—is necessary for the normal function of the nervous system and hematopoiesis. The entire path of cobalamin from intake to entry into the systemic circulation is shown in Figure 1. Vitamin B_{12} in foods is mostly bound to proteins. In the stomach, it is released by the action of hydrochloric acid and pepsin, after which it binds to

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R-protein. In the duodenum, pancreatic enzymes break down the complex with R-protein, which enables the binding of vitamin B_{12} to the intrinsic factor synthesized by the parietal cells of the stomach (Green et al., 2017; Vincenti et al., 2021; Čerkez Habek et al., 2023). The complex of intrinsic factor and vitamin B_{12} is absorbed in the distal ileum via specific receptors on enterocytes. After entering the portal circulation, most of the absorbed vitamin is bound to transcobalamin I and transported to the liver, while transcobalamin II enables transport to other tissues, where cobalamin is converted into its active coenzyme forms (Kirin, 2016; Krpan, 2024). The basic mechanism of absorption depends on the functionality of the receptor, but at an intake of ≥30 μg, passive diffusion also occurs, by which approximately 1–3% of the free vitamin is absorbed (Pawlak et al., 2013). The total absorption ranges between 11% and 65%, depending on the dose and the availability of receptors, whereby a small proportion of inactive analogues can be absorbed by passive diffusion (Nušinović, 2015). Vitamin B_{12} is predominantly stored in the liver and muscles, with its biological half-life estimated to be 1–4 years (Stahl and Heseker, 2007).

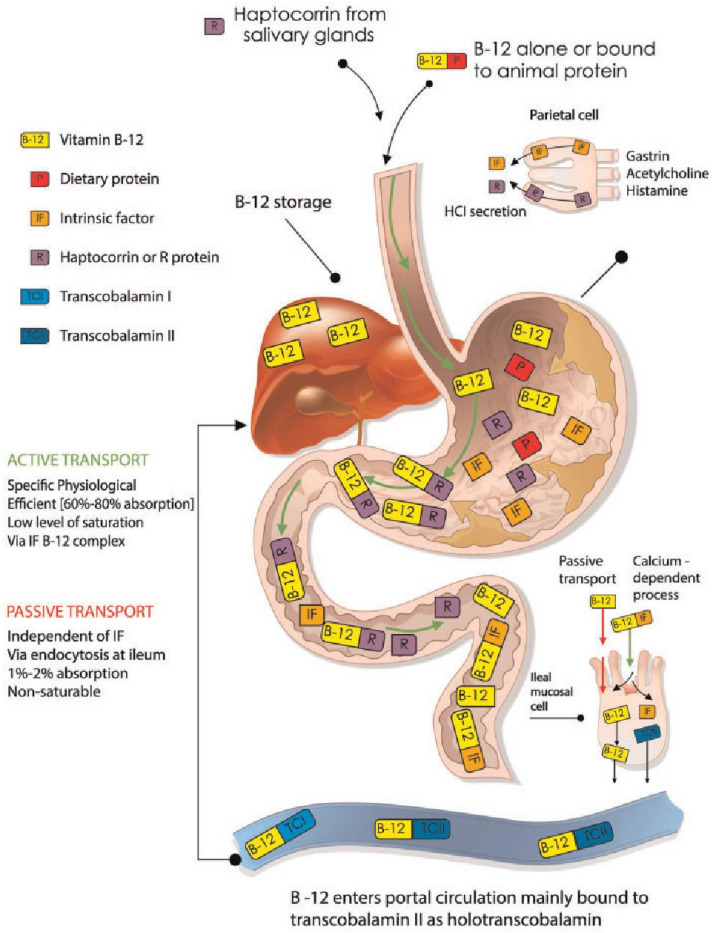

Figure 1. Mechanism of absorption and metabolism of vitamin B_{12} in the body (Brito et al., 2018)

Dietary sources of B_{12} and recommended intake

Vitamin B_{12} is present almost exclusively in foods of animal origin, while plant foods contain only minimal or inactive forms of cobalamin (Watanabe, 2007). In ruminants, vitamin B12 is synthesized by the action of microorganisms in the digestive system, and the amount of vitamin produced depends primarily on the intake of cobalt in the animal's diet (Kawashima et al., 1997; Ortigues-Marty et al., 2005). The most important dietary sources include liver, muscle tissue, fish, milk, and fermented milk products. As previously mentioned, cobalamin content in these foods depends on the diet and breeding conditions of the animals,including the technological processing of the food (Gille and Schmid, 2015). Their regular consumption is associated with higher serum concentrations of vitamin B12 (Barr et al., 2000; Stabler and Allen, 2004; Villamor et al., 2008;

Brouwer-Brolsma et al., 2015). However, different foods contain different forms of cobalamin, e.g. adenosylcobalamin and hydroxycobalamin are present in meat and fish, while methylcobalamin is mainly found in fermented milk products (Nušinović, 2015; Sobczyńska-Malefora et al., 2021). The recommended daily intake of vitamin B12 differs according to age and physiological needs and is defined by the guidelines of various institutions. According to the US Institute of Medicine (IOM, 1998) and the European Food Safety Agency (EFSA, 2015), the recommended values are several micrograms per day, which is sufficient to maintain normal metabolism and prevent deficits (Table 1).

Table 1. Recommended daily intakes of vitamin B_{12} (IOM, 1998; EFSA, 2015)

Ages	IOM (USA) RDA	EFSA (EU) PRI
Babies 0–6 months old	0.4 µg	0.4 µg
Babies 7–12 months old	0.5 µg	0.5 µg
Children 1–3 years old	0.9 µg	1.5 µg
Children 4–8 years old	1.2 µg	1.5 µg
Children 9–13 years old	1.8 µg	4.0 µg
Adolescents 14–18 years old	2.4 µg	4.0 µg
Adults (19+ years old)	2.4 µg	4.0 µg
Pregnant women	2.6 µg	4.5 µg
Nursing mothers	2.8 µg	5.0 µg

Bioavailability of B_{12}

The proportion of the ingested amount of cobalamin that is absorbed and becomes available for metabolic processes is bioavailable. It depends on the origin of the nutrient, the functional state of the digestive system, interactions with drugs and other nutrients, age, and food preparation methods. The content of vitamin B_{12} in meat varies depending on the age of an animal; younger animals have lower levels due to insufficiently developed storage capacities, so veal and lamb contain fewer amount than beef and mutton (Williams, 2007).

Bioavailability from chicken meat is about 61–65%, depending on the amount of intake (Doscherholmen et al., 1978). The concentration of the vitamin is also affected by the proportion of lipids in the meat, whereby lean parts contain more B12 (Ortigues-Marty et al., 2006). Thermal processing of meat products causes losses of 10% to 40%, which is particularly pronounced at high temperatures (Molonon et al., 1980; Gille and Schmid, 2015). Fish is a stable source of cobalamin, with minimal losses (2.3–14.8%) regardless of the method of preparation (Nishioka et al., 2006). In contrast, liver pate, although very rich in B_{12}, has low absorption (~10%) (Scott, 1997).

Absorption from eggs is extremely low (3.7–9.2%) due to the low availability of protein-bound vitamins (Squires and Naber, 1992). In milk, the concentration is stable, but cooking and microwave processing can cause losses of 30–50%, while pasteurization does not change the content of cobalamin (Watanabe et al., 1998). Fermentation of milk with bacteria *Lactobacillus bulgaricus* and *Streptococcus thermophilus* reduces B12 content, while *Propionibacterium shermanii* increases its concentration (Arkbåge et al., 2003).

The average absorption of vitamin B12 from a mixed diet is 35–40%, and it decreases with increased consumption of cobalamin per meal due to saturation of the absorption mechanism (Ströhle et al., 2019; Krpan, 2024).

Laboratory diagnostics of B_{12} status

Laboratory diagnosis of vitamin B_{12} deficiency is complex because neurological symptoms occur in 75–90% of patients, and in a quarter, those are the only symptoms, without macrocytic anemia (FNB, 1998). The basic assessment relies onserum vitamin B_{12}, with values <148 pmol/L (<200 pg/mL) considered the threshold for deficiency. The microbiological test is the most specific, while radiodilution and chemiluminescence offer faster, but less reliable results (Nemet, 2000). In pernicious anemia, a markedly reduced urinary excretion of vitamin B12 is a direct sign of low absorption in the gut (Sokoloff et al., 1952).

Considering possible falsely low values, simultaneous determination of folate is recommended (Vlasveld, 2003), especially since folate deficiency can mimic the clinical picture of B_{12} deficiency. Falsely reduced values also occur in multiple myeloma and in women using oral contraceptives (Shojania, 1982). Homocysteine and methylmalonic

acid (MMA) are typically elevated, while only homocysteine is elevated in folate deficiency. Autoimmune gastritis is accompanied by increased gastrin and decreased pepsinogen (Brinar, 2009).
Hematologic findings include macrocytic anemia (MCV 100–150 fL), reticulocytopenia, leukopenia, thrombocytopenia, with elevated LDH, bilirubin, iron, and ferritin, and decreased haptoglobin. In the bone marrow, there is a hypercellularity with megaloblastic hematopoiesis (Vrhovac et al., 2008). In the differential diagnosis, it is necessary to exclude infections (HIV-1, neurosyphilis) (Balt, 2000; Pandey, 2011), demyelinating diseases such as multiple sclerosis (Miller et al., 2005), and metabolic disorders (copper, vitamin E deficiency, exposure to N_2O).

Assessment of vitamin B_{12} absorption

The vitamin B_{12} absorption assessment has evolved from traditional radioisotope methods to safer and more accurate techniques. The Schilling test, although historically important, is rarely used today due to limitations in assessing protein-bound B_{12} and radioactivity (Vuk, 2016). Similar limitations are present in the yolk absorption test.
More modern approaches include CobaSorb, a non-radioactive method that measures the increase in holotranscobalamin after low oral doses and is effective in detecting malabsorption (Brito et al., 2018). A highly sensitive but technically demanding alternative is the ^{14}C-labeled B_{12} assay analysed by accelerator mass spectrometry (Brito et al., 2018).
In addition to diagnostics, strategies to improve absorption are being developed, including preparations linked to transport proteins or peptides, excipients such as SNAC, and probiotic and fermented products as potential sources and modulators of bioavailability (Brito et al., 2018).

Vitamin B_{12} status and neurological manifestations of deficit

The prevalence of vitamin B_{12} deficiency varies significantly among populations and is most often associated with limited intake of animal foods, economic and social factors, as well as dental and gastrointestinal problems that reduce intake or absorption (Baik and Russell, 1999). The elderly and patients with chronic malabsorption are the most at-risk groups. Epidemiological data consistently confirm the high prevalence of deficits in elderly people. In Nepal, 33.5% of $\geq$ 60-year-olds had a laboratory-confirmed deficiency (Gjawali et al., 2023), while in India the prevalence was 42.3%, especially pronounced in women over 75 years of age (Kumar et al., 2021). Data from the US (NHANES 2023) indicate a prevalence of approximately 20% among people over 60 years of age (CDC, 2023). In Croatia, Miškulin et al. (2014) found a deficit in 7.1% of the respondents, all of whom had concurrent symptoms of depression. Subclinical vitamin B_{12} deficiency, defined by reduced biochemical values without hematological or neurological manifestations, has particular clinical importance because it can progress to serious neurological damage before the appearance of clear symptoms (Carmel, 2011 b). Evidence suggests that subclinical deficiency is associated with cognitive decline, dementia, and depression (Bailey et al., 2015), and is most common in the elderly due to reduced absorption and metabolic changes (Allen et al., 2018). Evidence supports the need to introduce routine screening for vitamin B_{12} deficiency in ederly (Wong, 2015).
Unlike deficiency, vitamin B_{12} excess is much less common, but its clinical significance is becoming increasingly clear. Elevated serum concentrations do not usually reflect excessive dietary intake, but can be a marker of serious pathological conditions, including liver disease, renal failure, hematological disorders, and malignant diseases (Zulfiqar et al., 2019). In the geriatric population, excess is a strong predictor of mortality and is associated with more pronounced comorbidities. In a French geriatric acute care setting, 25.3% of hospitalized elderly patients had elevated levels of vitamin B_{12}, which were associated with acute renal failure, liver disease, and solid neoplasia (Zulfiqar et al., 2017).
Lack of vitamin B_{12} is most often the result of reduced intake, impaired absorption, or increased consumption of vitamins, whereby the elderly population is at the highest risk. Reduced intake occurs especially in people who do not consume products of animal origin;therefore, the prevalence of the deficit reaches more than 60% in vegans and about 40% in lacto- and ovo-lactovegetarians (Herrmann and Obeid, 2017). In the elderly population, primary causes include age-related changes in the gastrointestinal tract, such as hypochlorhydria and atrophic gastritis (Baik and Russell, 1999; Andrès et al., 2004), and pernicious anemia caused by a deficiency of intrinsic factor required for the absorption of vitamin B_{12}. Chronic gastrointestinal diseases, including Crohn's disease, celiac disease, and the post-gastrectomy condition, further impair the absorption of vitamin B_{12} (Allen, 2008). The status of vitamin B_{12} is significantly affected by numerous drugs, among which proton pump inhibitors and H_2-blockers (Lam et al., 2013),

metformin (de Jager et al., 2010; Ting et al., 2006), colchicine (Penniston et al., 2010), and anticonvulsants (Selby et al., 1998) stand out. Less common but clinically significant causes include parasitic infections such as *Diphyllobothrium latum*, which competitively consume vitamin B_{12} (Allen, 2009), and malnutrition and adverse social determinants of health, including poor appetite, dental problems, and depression in the elderly (Herrmann and Obeid, 2017). Autoimmune diseases (e.g., Hashimoto thyroiditis, vitiligo) and chronic inflammatory diseases can further impair the absorption and metabolism of vitamin B_{12} (Andrès et al., 2004), whereas liver and kidney diseases reduce the body's ability to store and recycle vitamin B_{12} (Herrmann and Obeid, 2017). Understanding a wide range of etiological factors is crucial for timely diagnosis and multidisciplinary management of subclinical and clinical vitamin B deficiency in the elderly population.

Neurological changes often appear independently or in combination with other symptoms; they are present in 4–50% of patients, and without timely vitamin supplementation, demyelination, axonal degeneration, and neuronal death occur, resulting in permanent damage to the nervous system. Table 2 shows the most common symptoms associated with B_{12} deficiency.

Table 2. Overview of symptoms of vitamin B_{12} deficiency in the peripheral, central, and autonomic nervous system

Symptom	Description of symptoms	Referrence
Peripheral neuropathy	Tingling, prickling, burning in hands and feet (symmetrical)	Allen et al., 2018; Carmel, 2011 b
Ataxia	Unsteady gait, balance disorder (especially in the dark)	Baik & Russell, 1999;Langan & Goodbred, 2017
Spasm	Stiffness and increased muscle tone	Mikkelsen & Apostolopoulos, 2018; Carmel, 2011b
Muscle weakness	Usually in the lower extremities	Baik & Russell, 1999; Mikkelsen & Apostolopoulos, 2018
Subacutecombined degeneration	Degeneration of the posterior and lateral columns of the spinal cord	Carmel, 2011b; Langan & Goodbred, 2017
Optical neuropathy	Blurred vision, central scotoma, reduced colour vision	Calderón-Ospina & Nava-Mesa, 2020; Allen et al., 2018
Positive Babinski reflex	Pathological reflex - a sign of upper motor neuron lesion	Baik & Russell, 1999; Carmel, 2011a
Autonomousdysfunction(rarely)	Orthostatic hypotension, urination disorders	Baik & Russell, 1999; Calderón-Ospina & Nava-Mesa, 2020

Aging, itself causes changes in the gastrointestinal tract which directly imapacts vitamin B_{12} absoprtion – from reduced consumption of meat to dental issues, to lower acidity in the stomach and atrophy of the stomach that affects intrinsic factor, to impaired motility. When combined with underlaying chronic health issues and (multi)mediaction use, the risk of deficiency among elderly is especially high (Mouchaileh, 2023).

Concluding remarks

Available literature proves that vitamin B_{12} deficiency is frequent, but insufficiently recognized in routine clinical practice among elderly. This may be due to methodological differences between studies, including the definition of deficiency (serum B_{12} cut-offs), the biomarkers assessed (serum B_{12}, holotranscobalamin, methylmalonic acid), participant selection, age groups, as well as geographic, and socio-economic factors.

While some studies focus on laboratory-confirmed deficiencies in older adults, others address subclinical deficiency or populations with specific risk factors, such as users of metformin or proton pump inhibitors. This heterogeneity complicates direct comparison of results and underscores the need for standardized protocols in future research. Currently, there is no recommendation for mass screening for vitamin B12 in the elderly, and given the increased number of elderly in the global population (WHO, 2025) and high burden of neurodegenerative disorders (GBD 2021 Nervous System Disordes Collaborators, 2024), there is an evident need to improve current practice. Based on the literature reviewed, we found sparse evidence from long-term studies that focused on early detection of vitamin B_{12} deficiency, and targeted replacement to prevent neurological damage among elderly. Particularly underexplored areas include interactions of vitamin B_{12} with medications and nutritional factors, as well as strategies to optimize early detection of subclinical deficiency in older

populations. Because symptoms often develop gradually and can be nonspecific including peripheral neuropathies, balance disturbances, cognitive decline, and mood changes the deficit is often misattributed to aging processes or other comorbidities. Additionally, the fact that neurological changes can occur even at serum levels of vitamin B_{12} within the reference range highlights the limitations of conventional diagnostic criteria. In this context, the use of more specific biomarkers, such as holotranscobalamin and methylmalonic acid, represents a significant advance in assessing the functional status of B_{12}. These markers enable early detection of cobalamin metabolism disorders, especially in risk groups such as the elderly, people with chronic gastrointestinal diseases, patients after bariatric procedures, and users of metformin and proton pump inhibitors. Including these parameters in diagnostic algorithms can significantly improve the timely recognition of deficits and reduce the risk of irreversible neurological consequences.

It is necessary to educate healthcare professionals about the need for early screening for vitamin B_{12} status, while older adults, especially those with chronic conditions known to affect vitamin B_{12} bioavailability need specific education on risks of low vitamin B_{12} status and its neurological consequences. The proactive implementation of targeted supplementation and preventive strategies, based on insights from the reviewed studies, may reduce the incidence of neurological complications, improve quality of life in older adults, and optimize healthcare system resources.

References

Allen, L.H. (2008): Causes of vitamin B12 and folate deficiency, *Food Nutr. Bull.* 29 (2), S20–S34.

Allen, L.H., Miller, J.W., de Groot, L., Rosenberg, I.H., Smith, A.D., Refsum, H., Raiten, D.J. (2018): Biomarkers of Nutrition for Development (BOND): vitamin B12 review, *J. Nutr.* 148 (Suppl. 4), 1995S–2027S.

Andrès, E., Loukili, N.H., Noel, E., Kaltenbach, G., Abdelgheni, M.B., Perrin, A.E., Schlienger, J.L. (2004): Vitamin B12 (cobalamin) deficiency in elderly patients, *Can. Med. Assoc. J.* 171 (3), 251–259.

Arkbåge, K., Witthöft, C.M., Fondén, R., Jägerstad, M. (2003): Retention of vitamin B12 during manufacture of six fermented dairy products using a validated radio protein-binding assay, *Int. Dairy J.* 13, 101–109.

Baik, H.W., Russell, R.M. (1999): Vitamin B12 deficiency in the elderly, *Annu. Rev. Nutr.* 19, 357–377.

Bailey, R.L., West, K.P., Black, R.E. (2015): The epidemiology of global micronutrient deficiencies, *Ann. Nutr. Metab.* 66 (2), 22–33.

Balt, C.A. (2000): An investigation of the relationship between vitamin B12 deficiency and HIV infection, *J. Assoc. Nurses AIDS Care* 11 (1), 24–28, 31–35.

Barr, S.I., McCarron, D.A., Heaney, R.P., Dawson-Hughes, B., Berga, S.L., Stern, J.S., Oparil, S. (2000): Effects of increased consumption of fluid milk on energy and nutrient intake, body weight, and cardiovascular risk factors in healthy older adults, *J. Am. Diet. Assoc.* 100 (7), 810–817

Brinar, V. (2009):*Neurologija za medicinare.* 1. izd. Zagreb, HR: Medicinska naklada.

Brito, A., Habeych, E., Silva-Zolezzi, I., Galaffu, N., Allen, L.H. (2018): Methods to assess vitamin B12 bioavailability and technologies to enhance its absorption, *Nutr. Rev.* 76 (10), 778–792.

Brouwer-Brolsma, E.M., Dhonukshe-Rutten, R.A., van Wijngaarden, J.P., Zwaluw, N.L., van de Velde, N., de Groot, L.C. (2015): Dietary sources of vitamin B-12 and their association with vitamin B-12 status markers in healthy older adults in the B-PROOF Study, *Nutrients* 7 (9), 7781–7797.

Bundalo, D., Srkalović Imširagić, A. (2013): Vitamini skupine B u psihijatriji, *Medicus* 22 (2), 139–144.

Calderón-Ospina, C.A., Nava-Mesa, M.O. (2020): B vitamins in the nervous system: Current knowledge of the biochemical modes of action and synergies of thiamine, pyridoxine, and cobalamin, *CNS Neurosci. Ther.* 26 (1), 5–13.

Carmel, R. (2011a): Biomarkers of cobalamin (vitamin B-12) status in the epidemiologic setting: A critical overview of context, applications, and performance characteristics of cobalamin, methylmalonic acid, and holotranscobalamin II, *Am. J. Clin. Nutr.* 94, 348S–358S.

Carmel, R. (2011b): Nutritional anemias and the elderly, *Baillière's Best Pract. Res. Clin. Haematol.* 13 (3), 623–639.

CDC (2023): National Health and Nutrition Examination Survey (NHANES) data. Centers for Disease Control and Prevention. (Web izvor; potrebno dodati datum pristupa ako želiš potpunu citaciju.)

Clarke, R., Sherliker, P., Hin, H., Nexo, E., Hvas, A.M., Schneede, J., Birks, J., Ueland, P.M., Emmens, K., Scott, J.M., Molloy, A.M., Evans, J.G. (2007): Detection of vitamin B12 deficiency in older people by measuring vitamin B12 or the active fraction of vitamin B12, holotranscobalamin, *Clin. Chem.* 53 (5), 963–970.

Čerkez Habek, J. (ur.) i suradnici; (2023): *Medicina prehrane.* Zagreb, HR: Medicinska naklada.

de Jager, J., Kooy, A., Lehert, P., Wulffelé, M.G., van der Kolk, J., Bets, D., Verburg, J., Donker, A.J.M., Stehouwer, C.D.A. (2010): Long-term treatment with metformin in patients with type 2 diabetes and risk of vitamin B-12 deficiency: randomized placebo-controlled trial, *BMJ* 340, c2181.

Doscherholmen, A., McMahon, J., Ripley, D. (1978): Vitamin B-12 assimilation from chicken meat, *Am. J. Clin. Nutr.* 31, 825–830.

EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) (2015): Scientific opinion on Dietary Reference Values for vitamin B12. *EFSA J.* 13 (7), 4150. Food and Nutrition Board, Institute of Medicine (1998): Vitamin B12. In: *Dietary Reference Intakes: Thiamin, Riboflavin, Niacin, Vitamin B6, Vitamin B12, Pantothenic Acid, Biotin, and Choline.* Washington D.C., USA: National Academy Press, 306–356.

GBD 2021 Nervous System Disorders Collaborators. (2024). Global, regional, and national burden of disorders affecting the nervous system, 1999-2021: a systematic analysis for the Global Burden of Disease Study 2021, *Lancet Neuolorgy*, 23(4), 344-381.

Gille, D., Schmid, A. (2015): Vitamin B12 in meat and dairy products, *Nutr. Rev.* 73 (2), 106–115.

Green, R., Allen, L.H., Bjørke-Monsen, A.L., Brito, A., Guéant, J.L., Miller, J.W., Molloy, A.M., Nexo, E., Stabler, S., Toh, B.H., Ueland, P.M., Yajnik, C. (2017): Vitamin B12 deficiency, *Nat. Rev. Dis. Primers* 3, 17040.

Gyawali, P., Bhatt, R.D., Karmacharya, R.M., Pant, V., Khadka, A. (2023): High burden of vitamin B12 deficiency among adults and elderly, *J. Nepal Health Res. Counc.* 20 (3), 702–707.

Herbert, V. (1988): Vitamin B12: Plant sources, requirements, and assay, *Am. J. Clin. Nutr.* 48 (3 Suppl), 852–858.

Herrmann, W., Obeid, R. (2017): Causes and early diagnosis of vitamin B12 deficiency, *Dtsch. Arztebl. Int.* 114 (47), 680–685.

Institute of Medicine (IOM) (1998): *Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin, and Choline.* Washington, DC: National Academy Press.

Karakaš, S., Antonić, B. (2017): *Epidemiologija ishrane sa metodologijom uzorkovanja i procjene rizika.* Graforad, str. 502.

Kawashima, T., Henry, P.R., Ammerman, C.B., Littell, R.C., Price, J.D. (1997): Bioavailability of cobalt sources for ruminants. 2. Estimation of the relative value of reagent grade and feed grade cobalt sources from tissue cobalt accumulation and vitamin B12 concentrations, *Nutr. Res.* 17, 957–974.

Kirin, S. (2016): *Neurološke smetnje uslijed manjka vitamina B12* (diplomski rad). Rijeka: Sveučilište u Rijeci, Medicinski fakultet.

Krpan, D. (2024): *Prehrambeni unos vitamina B12 među dobrovoljnim davateljima krvi* (diplomski rad). Osijek: Sveučilište Josipa Jurja Strossmayera u Osijeku, Prehrambeno-tehnološki fakultet.

Kumar, A., Sharma, P., Singh, S. (2021): Vitamin B12 deficiency in elderly Indian population: A cross-sectional study, *Front. Public Health* 9, 707036.

Lam, J.R., Schneider, J.L., Zhao, W., Corley, D.A. (2013): Proton pump inhibitor and histamine-2 receptor antagonist use and vitamin B12 deficiency. *JAMA* 310 (22), 2435–2442.

Langan, R.C., Goodbred, A.J. (2017): Vitamin B12 deficiency: Recognition and management, *Am. Fam. Physician* 96 (6), 384–389.

Leishear, K., Boudreau, R.M., Studenski, S.A., Ferrucci, L., Rosano, C., de Rekeneire, N., Houston, D.K., Kritchevsky, S.B., Schwartz, A.V., Vinik, A.I., Hogervorst, E., Yaffe, K., Harris, T.B., Newman, A.B. (2012): Relationship between vitamin B12 and sensory and motor peripheral nerve function in older adults, *J. Am. Geriatr. Soc.* 60 (6), 1057–1063.

Mathew, A.R., Di Matteo, G., La Rosa, P., Barbati, S.A., Mannina, L., Moreno, S., Tata, A.M., Cavallucci, V., Fidaleo, M. (2024): Vitamin B12 deficiency and the nervous system: Beyond metabolic decompensation, *Int. J. Mol. Sci.* 25 (1), 590.

Miškulin, M., Kristić, M., Vlahović, J. (2014): Vitamin B12 deficiency and depression in elderly: Cross-sectional study in Eastern Croatia, *J. Health Sci.* 4 (3), 143–148.

Mikkelsen, K., Apostolopoulos, V. (2018): B vitamins and ageing. In: Harris, J., Korolchuk, V. (ur.): *Biochemistry and Cell Biology of Ageing: Part I Biomedical Science.* Subcellular Biochemistry, 90, 451–470. Springer, Singapore.

Molonon, B.R., Bowers, J.A., Dayton, A.D. (1980): Vitamin B12 and folic acid content of raw and cooked turkey muscle, *Poult. Sci.* 59, 303–307.

Moore, E., Mander, A., Ames, D., Carne, R., Sanders, K., Watters, D. (2012): Cognitive impairment and vitamin B12: A review, *Int. Psychogeriatr.* 24 (4), 541–556.

Mouchaileh, N. (2023). Vitamin B12 deficiency in older people: a practiacl approach to recognitiion and management, *Journal of Pharmacy Practice and Research*, 53, 350-358.

Nemet, D. (2000): Anemija i druge manifestacije nedostatka željeza, vitamina B12 i folata, *Medicus* 9 (1), 59–71.

Nishioka, M., Kanosue, F., Tanioka, Y., Miyamoto, E., Watanabe, F. (2006): Characterization of vitamin B12 in skipjack meats and loss of the vitamin from the fish meats by various cooking conditions, *Vitamins* (Japanese) 80, 507–511.

Nohr, D., Biesalski, H.K., Schümann, K. (2016): *Vitamin B12 – Mangel und Versorgung.* Stuttgart: Wissenschaftliche Verlagsgesellschaft.

Nušinović, E. (2015): *Proizvodnja vitamina B12 pomoću Pseudomonas denitrificans uz prihranjivanje betainom* (završni rad). Zagreb: Sveučilište u Zagrebu, Prehrambeno-biotehnološki fakultet.

Ortigues-Marty, I., Micol, D., Prache, S., Dozias, D., Girard, C.L. (2005): Nutritional value of meat: The influence of nutrition and physical activity on vitamin B12 concentrations in ruminant tissues, *Reprod. Nutr. Dev.* 45 (4), 453–467.

Ortigues-Marty, I., Thomas, E., Prévéraud, D.P., Girard, C.L., Bauchart, D., Durand, D., Peyron, A. (2006): Influence of maturation and cooking treatments on the nutritional value of bovine meats: Water losses and vitamin B12, *Meat Sci.* 73 (3), 451–458.

Pandey, S. (2011): Magnetic resonance imaging of the spinal cord in a man with tabes dorsalis, *J. Spinal Cord Med.* 34 (6), 609–611.

Pawlak, R., James, P.S., Raj, S., Cullum-Dugan, D., Lucus, D. (2013): Understanding vitamin B12, *Am. J. Lifestyle Med.* 7 (1), 60–65.

Penniston, K.L., Spadea, L., Covington, J., Pfeiffer, C., Reddy, K., Chou, C., Jacobsen, D.W. (2010): Vitamin B12 deficiency and colchicine-induced enteropathy, *Gastroenterology* 138 (5), 1841–1843.

Porter, K., Hoey, L., Hughes, C.F., Ward, M., McNulty, H. (2016): Causes, consequences and public health implications of low B-vitamin status in ageing, *Nutrients* 8, 725.

Scott, J.M. (1997): Bioavailability of vitamin B12, *Eur. J. Clin. Nutr.* 51 (Suppl 1), S49–S53.

Selby, L., George, S. (1998): Effect of antiepileptic drugs on serum vitamin B12 and folate, *Epilepsy Research* 31(3), 233–238.

Shojania, A. M. (1982): Oral contraceptives: Effect of folate and vitamin B12 metabolism, *Canadian Medical Association Journal* 126(3), 244–247.

Sobczyńska-Malefora, A., Delvin, E., McCaddon, A., Ahmadi, K. R., & Harrington, D. J. (2021): Vitamin B12 status in health and disease: A critical review. Diagnosis of deficiency and insufficiency—clinical and laboratory pitfalls, *Critical Reviews in Clinical Laboratory Sciences* 58(6), 399–429.

Sokoloff, M.F., Sanneman, E. H.,Beard, M.F. (1952): Urinary excretion of vitamin B12, *Blood* 7(2), 243-250.

Squires, M. W., Naber, E. C. (1992): Vitamin profiles of eggs as indicators of nutritional status in the laying hen: Vitamin B12 study, *Poultry Science* 71, 275–282.

Stabler, S. P., Allen, R. H. (2004): Vitamin B12 deficiency as a worldwide problem, *Annual Review of Nutrition* 24, 299–326.

Stahl, A., Heseker, H. (2007): Vitamin B12 (cobalamine), *Ernährungs Umschau* 54, 594–601.

Ströhle, A., Richter, M., González-Gross, M., Neuhäuser-Berthold, M., Wagner, K. H., Leschik-Bonnet, E., Egert, S., German Nutrition Society (DGE). (2019): The revised D-A-CH reference values for the intake of vitamin B12: Prevention of deficiency and beyond, *Molecular Nutrition & Food Research* 63(6), e1801178.

Ting, R. Z., Szeto, C. C., Chan, M. H., Ma, K. K., & Chow, K. M. (2006): Risk factors of vitamin B12 deficiency in patients receiving metformin, *Archives of Internal Medicine* 166(18), 1975–1979.

Vlasveld, L. T. (2003): Low cobalamin (vitamin B12) levels in multiple myeloma: A retrospective study, *Netherlands Journal of Medicine* 61(8), 249–252.

Villamor, E., Mora-Plazas, M., Forero, Y., Lopez-Arana, S., & Baylin, A. (2008): Vitamin B12 status is associated with socioeconomic level and adherence to an animal-food dietary pattern in Colombian school children, *The Journal of Nutrition* 138(7), 1391–1398.

Vrhovac, B., Jakšić, B., Reiner, Ž., Vucelić, B. (2008): *Interna medicina* (4th ed.). Naklada Ljevak.

Vuk, D. (2016): *Određivanje holotranskobalamina u ispitivanju statusa B12 vitamina* (Završni rad). Medicinski fakultet Osijek.

Watanabe, F., Abe, K., Fujita, T., Goto, M., Hiemori, M., Nakano, Y. (1998): Effects of microwave heating on the loss of vitamin B12 in foods, *Journal of Agricultural and Food Chemistry* 46(1), 206–210.

Watanabe, F., Bito, T. (2018): Vitamin B_{12} sources and microbial interaction, *Experimental biology and medicine* 243(2), 148–158.

Watanabe, F. (2007): Vitamin B12 sources and bioavailability, *Experimental Biology and Medicine* 232(10), 1266–1274.

WHO, World Health Organization. (2025): Ageing and health. Published: 1 October 2025. Available at: https://www.who.int/news-room/fact-sheets/detail/ageing-and-health

Williams, P. (2007): Nutritional composition of red meat, *Nutrition & Dietetics* 64(Suppl), S113–S119.

Wong, C. W. (2015): Vitamin B12 deficiency in the elderly: Is it worth screening?, *Hong Kong Medical Journal* 21(2), 155–164.

Zulfiqar, A. A., Sebaux, A., Dramé, M., & Andres, E. (2017): Hypervitaminemia B12 and malignant diseases: A cross-sectional study in an acute geriatric unit, *Annales de Biologie Clinique* 75(2), 193–203.

Zulfiqar, A. A., Andres, E., & Lorenzo Villalba, N. (2019): Hipervitaminosis B12: Our experience and a review, *Medicina* 79(5), 391–396.

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CURRENT STATUS AND THE NEED TO IMPROVE THE STANDARDIZATION OF ESSENTIAL OILS INTENDED FOR THE PRODUCTION OF FOOD SUPPLEMENTS

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review paper

Summary

The purpose of essential oil standardization is to ensure the quality, safety, and efficacy of their use in the production of food supplements, as well as in other pharmaceutical and cosmetic products. The current situation is characterized by fragmented standards, inconsistent quality control practices, and insufficient regulatory frameworks, which hinder their broader and safer application. Standardized essential oils or isolated active components most commonly peppermint, oregano, lemon, lavender, cinnamon, clove, basil, and others are frequently used in food supplement formulations. Due to potential toxicity at higher doses, they must be used with great caution. Numerous internal, national, and international standards are currently applied in defining the quality of essential oils. Standardization is most often based on pharmacopoeial monographs (e.g., Ph. Eur., USP), ISO standards (ISO/TC 54), the Codex Alimentarius, GRAS status (FDA, USA), and European regulations (EFSA). Regulation (EC) No 1334/2008 defines the use of flavorings, including essential oils, in food and food supplements, while Regulation (EC) No 1925/2006 regulates the addition of biologically active substances to food. Manufacturers of food supplements, in accordance with GMP and HACCP principles, must meet clearly defined requirements related to essential oils. Although certain standards and statuses, such as ISO and GRAS, exist, they still do not cover the full complexity and variability of essential oils as functional components. The aim of this paper is to define the current status and the need to improve the standardization of essential oils intended for the production of food supplements.

Keywords: essential oils, standardization, food supplements

Introduction

Essential oils (EOs) are complex mixtures of volatile bioactive compounds, such as monoterpenes, sesquiterpenes, phenols, and esters, which contribute to their pharmacological properties. Their chemical composition depends on the plant species, phenological stage, geographical origin, climate, cultivation method, and extraction technique (Bakkali et al., 2008; Turek and Stintzing, 2013). Their use in food supplements has been increasing due to their potential benefits for immunity, digestion, mood, and stress relief (da Silva Bomfim et al., 2020). However, the market faces major challenges, including fragmented standards, a lack of harmonization, and insufficient quality regulation (Petina, 2023). Variability in plant species, geographical origin, and extraction methods results in inconsistent quality and composition, which poses a challenge for ensuring safety, efficacy, and regulatory oversight (da Silva Bomfim et al., 2020; Petina, 2023).

Standardization aims to ensure consistent quality, safety, and efficacy of essential oils in food supplements. Essential oils generally contain primary, secondary, and trace components, leading to significant batch-to-batch variability. There are risks of toxicity associated with certain compounds, such as eugenol or menthol, when used in high doses. Additionally, adulteration (e.g., dilution with cheaper oils, addition of synthetic additives) remains common on the global market. Therefore, it is necessary to establish standardized protocols for sourcing, manufacturing, analysis, and application to protect public health (Chemat et al., 2020).

The aim of this paper is to provide a critical overview of key issues in the standardization of essential oils intended for food supplements, clarify the existing regulatory and technical framework, and highlight the need for improvement and harmonization of standards.

Chemical nature and bioactivity of essential oils for the production of food supplements

Essential oils show pronounced variability due to differences in botanical origin, geographical and climatic conditions, and extraction methods. Active components determine their therapeutic properties but also their potential for toxic reactions when consumed in high doses (Burt, 2004). For example,

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menthol—the main constituent of peppermint oil—has calming effects, but excessive intake may cause irritation.

The main chemical groups present in essential oils include monoterpenes (e.g., limonene, pinene), sesquiterpenes (e.g., β-caryophyllene), phenols (e.g., thymol, carvacrol), aldehydes, ketones, esters, and oxidized derivatives. Essential oils may contain:

- primary components (20–95%) – e.g., menthol in peppermint oil
- secondary components (1–20%) – contribute to synergistic effects
- trace components (<1%) – may exert strong biological activity despite low concentrations (da Silva Bomfim et al., 2020)

Due to their lipophilicity, bioactive molecules from essential oils readily cross cell membranes and exhibit diverse biological activity, including antimicrobial (bacteria, viruses, fungi), anti-inflammatory and antioxidant effects, immunomodulatory, sedative, and carminative actions, as well as stimulation of gastric secretions and bile flow, which supports digestion (Chemat et al., 2020).

Depending on the dose, composition, and route of administration, essential oils may function as beneficial dietary supplements or, in some cases, as potential toxicants. For example, high concentrations of eugenol may cause cytotoxicity, whereas limonene and linalool show good tolerability at low oral doses (European Commission, 2008).

In supplement formulation, essential oils are used because of their potential functional benefits (e.g., digestive and immune effects), aromatic properties that facilitate intake, and synergistic action with other active substances (e.g., vitamins, probiotics). Due to their complex and variable nature, ensuring a standardized chemical profile through methods such as GC-MS (gas chromatography–mass spectrometry) and chemometric analysis is essential for their safe and effective application in food supplements (Pavoni et al., 2020). Essential oils in food supplements represent one of the fastest growing areas of functional and natural nutrition. Their use has expanded from aromatherapy into clinical nutrition due to bioactive compounds with anti-inflammatory, antimicrobial, digestive, and sedative effects (EFSA, 2021; Kasper et al., 2010). They are used to support immunity, digestion, mental health, and circulation (EFSA, 2021). Advances in technology have enabled microencapsulation, adsorption, and complexation of essential oils with cyclodextrins to enhance stability and facilitate processing into conventional forms—tablets and capsules (Gharsallaoui et al., 2007; Szente and Szejtli, 2004). Demand for plant-based and natural supplements, particularly within clean-label, EU, U.S., halal, and kosher markets, is increasing (Halal Control, 2022; OU Kosher, 2023).

Essential oils are increasingly combined with vitamins, minerals, and probiotics to create synergistic formulations supporting immune, nervous, and digestive functions. They show potential for therapeutic supplementation in conditions such as IBS, stress, insomnia, and menopause (Kasper et al., 2010). Nevertheless, challenges include the need for standardization, clinical trials, and precise labelling of constituents (EFSA, 2021).

Dry extracts of essential oils in food supplements

Dry extracts of essential oils represent processed forms adapted for use in food supplements. Although technically not pure essential oils in their original state, they retain active components and provide simpler, more stable, and safer oral administration. Dry extracts rely on specific production technologies such as microencapsulation, adsorption, cyclodextrin complexation, and dry emulsions.

In microencapsulation, essential oil is enclosed within a protective coating via spray-drying and converted into powder. In adsorption, the oil is bound to carriers such as silica and transformed into powder. In cyclodextrin complexation, active molecules are incorporated into cyclic molecules that protect them. In dry emulsions, stable emulsions of essential oils are dried and used as granulate (Gharsallaoui et al., 2007; Szente and Szejtli, 2004). Advantages of dry forms include improved stability against light, oxygen, and heat, reduced intensity of aroma and taste, easier dosing and formulation into capsules or tablets, increased safety for oral intake, and suitability for industrial processing and transport. However, dry forms may contain only part of the components from the original oil, are not considered pure essential oils according to ISO 9235, and must be clearly labelled as essential oil extracts (e.g., microencapsulated lavender extract) (ISO, 2013). Examples in practice include lavender oil tablets (e.g., Silexan) for anxiety and stress, oregano capsules for antibacterial and immunostimulatory effects, and powdered preparations containing lemon oil in beverages for immune support (Kasper et al., 2010).

Dry extracts of essential oils constitute functionally valuable forms for food supplements. Their safety, stability, and convenient use make them an increasingly frequent choice for manufacturers. It is

essential that they are clearly declared, properly dosed, and compliant with technological and regulatory standards.

Current status of regulation and standards

Standardization of essential oils refers to a set of controlled procedures and measures ensuring that an essential oil has a known and consistent chemical composition, meets qualitative and quantitative specifications, and is safe, effective, and suitable for its intended use (food, cosmetics, pharmaceuticals). Standardization includes identification of the botanical source (Latin name, plant part, origin), definition of the chemical profile (GC–MS analysis), establishing limits for key components (e.g., ≥30% linalool in lavender), restricting undesirable compounds (e.g., thujone, safrole), control of physical parameters (density, refractive index), and documentation on cultivation, extraction, and storage (ISO, 2013; EFSA, 2021). Example: according to ISO 3515 (ISO, 2002), standardized lavender oil must contain 30–45% linalyl acetate, 25–38% linalool, and <0.5% camphor.

Standardization is essential because it ensures consistent functionality in every batch, eliminates toxic components, enables regulatory compliance with legal requirements, ensures clinical efficacy with known doses of active compounds, and provides a market advantage through higher value and consumer trust. Standardization of essential oils is crucial for the safety, quality, and regulatory compliance of products. It enables the use of essential oils in food, supplements, and pharmaceutical preparations in a scientifically substantiated manner.

Numerous challenges exist in the standardization and quality assurance of essential oils in food supplements. Variability in composition complicates consistent bioactivity (Petina, 2023), adulteration with cheaper substitutes is common (Smith et al., 2023), GC–MS analyses are not standardized across laboratories (Pavoni et al., 2020), formulations may be unstable if not properly developed (Saka and Chella, 2020), regulatory approaches are not globally harmonized (FDA, 2021; European Commission, 2008), and transparency regarding raw material origin is often lacking (Compliance Gate, 2025).

Standardization is carried out according to the following frameworks:

- ISO standards 9235 and 3515 – international standards;
- Ph. Eur., USP, BP – pharmacopoeial standards;
- Codex Alimentarius – food guidelines;
- EFSA and FDA – safety assessments (FAO/WHO, 2001; EFSA, 2021).

ISO standards

ISO standards provide technical guidelines but are voluntary and often not integrated into national legislation for food supplements. Their value lies in ensuring consistency between laboratories, but they do not define permissible limits or health claims.

The technical committee ISO/TC 54 is responsible for developing international standards related to the analysis, specification, and labelling of essential oils (ISO, 2025a). The committee operates within ISO headquartered in Spain and collaborates with organizations such as Codex Alimentarius, FAO, WHO, and industry associations such as IFEAT. To date, more than 160 international ISO standards have been published for specific essential oils, including rosemary oil, grapefruit oil, sandalwood oil, and standards for determining trace benzene in essential oils (ISO, 2025b; ISO, 2024a; ISO, 2024b).

ISO has developed technical specifications standardizing ingredient terminology (ISO, 2025c). The standards define methods such as GC–MS chromatographic fingerprinting (ISO 11024-1:1998), optical rotation, relative density, freezing point, and packaging labelling requirements. Implementation of these standards ensures laboratory consistency in quality assessment but does not prescribe therapeutic doses, allowable concentrations, or health claims, limiting their regulatory value in the context of food supplements (Ibáñez, 2016).

Despite their scientific value, ISO standards face practical limitations. They are voluntary and not legally binding, leading many manufacturers to apply them inconsistently (Ibáñez, 2016). National legislations rarely incorporate ISO standards, further limiting their regulatory influence. Specialized essential oils often deviate from ISO profiles to meet market demands for aroma or price. It is recommended that ISO standards be harmonized with regulatory frameworks of EFSA, Ph. Eur., GRAS, and formally integrated into EU legislation for food supplements containing essential oils, making their application mandatory in industrial production (ISO, 2025a; ISO, 2024b).

Pharmacopoeias

Pharmacopoeias are official compendia of standards defining the quality, purity, identity, and analytical methods for medicinal substances, including essential oils when used for medicinal, cosmetic, or nutritional purposes. The two most important sources are:

- European Pharmacopoeia (Ph. Eur.) – published by the European Directorate for the Quality of Medicines (EDQM), legally binding in the EU.

- United States Pharmacopeia (USP) – recognized standard in the USA, mandatory for products on the U.S. market.

Both pharmacopoeias include documents defining chemical composition, identification methods, purity, and contaminant limits for specific oils. They contain monographs for individual essential oils with clearly defined purity criteria (Ph. Eur., 2023; USP, 2023). Monographs are fundamental in ensuring quality and identity, harmonizing manufacturing and batch control, and providing the legal basis for inspections and market authorization.

European Union regulations

EU Regulations No. 1334/2008 and 1925/2006 form the key legal frameworks for essential oils in food and dietary supplements. Although they recognize essential oils as flavourings, they do not define their therapeutic or functional status when used in food supplements (European Commission, 2008; 2006).
Essential oils are classified as "flavourings of plant origin". They are permitted exclusively as flavourings, without health claims or therapeutic indications. Regulation (EC) No. 1925/2006 regulates the addition of vitamins, minerals, and other substances with nutritional or physiological effects. Essential oils are not explicitly listed, and their functional use requires a specific EFSA assessment. These regulations prohibit claims such as “supports immunity” or “has antibacterial effects” unless previously approved by EFSA, creating legal uncertainty for manufacturers wishing to position essential oils as functional ingredients (Petina, 2023).

FDA regulation of essential oils in dietary supplements

In the United States, GRAS status allows the use of small amounts of oils but does not cover therapeutic doses (FDA, 2021). The Food and Drug Administration (FDA) regulates essential oils through several mechanisms depending on their intended use. Two key aspects include GRAS status and labelling/claims requirements (FDA, 2024).
Many essential oil constituents have GRAS status for use in food products, meaning they are considered safe when used in low concentrations as flavourings (FDA, 2024). Examples include menthol, eugenol, limonene, and linalool listed in 21 CFR §182.20 (CFR, 2024). GRAS status does not cover therapeutic use. If essential oils in dietary supplements are marketed with health claims, such claims must be scientifically substantiated, and manufacturers must include the appropriate FDA disclaimer (FDA, 2023).
Manufacturers intending to use essential oils in dietary supplements must:

- use only GRAS-recognized components,
- comply with labelling regulations and avoid unauthorized claims,
- implement good manufacturing practices (cGMP),
- ensure scientific evidence of safety and efficacy.

Codex Alimentarius and essential oils in food supplements

Codex Alimentarius is an international collection of standards and guidelines for food safety and quality, developed by FAO and WHO. Its purpose is to protect consumer health and facilitate international food trade (FAO/WHO, 2023).
Codex does not directly regulate essential oils as ingredients in food supplements but classifies them as flavourings and technical additives. Due to its voluntary nature, application varies widely among countries, posing challenges for manufacturers and global trade.

GAP and organic production standards

Good Agricultural Practices (GAP) represent a set of minimum principles applied during plant cultivation to ensure safety, quality, and traceability of raw materials (FAO, 2023). In essential oil production, GAP is crucial for maintaining chemical profiles, reducing pesticide residues, and ensuring hygienic conditions.
Organic production involves cultivation without synthetic pesticides, GMOs, chemical fertilizers, or growth regulators (IFOAM, 2022). In the EU, Regulation (EU) 2018/848 defines conditions for certified organic production of plants intended for distillation and use in food products.
Organic oils often carry certifications such as EU Organic, USDA Organic, Ecocert, or Soil Association, which increase their market value and consumer trust.
GAP ensures basic hygiene and safety standards, while organic farming represents an advanced certification system. For supplements containing essential oils, organic production offers higher quality and market acceptance (Turek and Stintzing, 2013; IFOAM, 2022).

Halal, Kosher and similar standards

The status of dietary supplements containing essential oils varies among world religions depending on dietary rules, ethical norms, and production processes. Such supplements may be acceptable in most religions if they meet criteria of purity, origin, and ethical production.

Halal and kosher certification is essential for the Muslim and Jewish populations. Other religions emphasize natural origin, non-violence, and the absence of alcohol or harmful additives.

Most common formulations of essential oils in food supplements

Formulations of essential oils in food supplements include softgel capsules, emulsions, nanoemulsions, tablets, capsules, oral dispersible powders, and sublingual sprays. Nanoemulsions show improved bioavailability (Saka and Chella, 2020), whereas powder products are more stable but may exhibit lower efficacy (da Silva Bomfim et al., 2020).

Vegetable oils and essential oils most commonly used in food products and supplements in Bosnia and Herzegovina and neighbouring countries are presented in the table below. All oils comply with EU regulations and Codex Alimentarius guidelines (EFSA, 2021; FAO/WHO, 2001).

Table 1. Commonly used essential oils in food supplements

Name of oil	Latin name	Effect / use
Peppermint oil	*Mentha piperita* / *arvensis*	Digestive, against bloating and nausea
Lavender oil	*Lavandula angustifolia*	Calming, anti-stress, for sleep
Lemon oil	*Citrus limonum*	Antioxidant, stimulant, supports immunity
Ginger oil	*Zingiber officinale*	Digestive, anti-inflammatory, circulation
Immortelle (helichrysum) oil	*Helichrysum italicum*	Antioxidant, cell protection, aromatic use
Basil oil	*Ocimum basilicum*	Digestive, antimicrobial
Rosemary oil	*Rosmarinus officinalis*	Cognitive support, antioxidant
Lemongrass oil	*Cymbopogon citratus*	Digestion, anti-inflammatory
Wild oregano oil	*Origanum vulgare*	Antibacterial, immunostimulant
Clove oil	*Syzygium aromaticum*	Antiseptic, analgesic, oral care
Black seed oil	*Nigella sativa*	Immune support, antioxidant
Anise oil	*Pimpinella anisum*	Digestion, against bloating
Coriander oil	*Coriandrum sativum*	Digestive, antioxidant
Cinnamon oil	*Cinnamomum zeylanicum*	Anti-inflammatory, glucose support
Thyme oil	*Thymus vulgaris*	Respiratory health, antimicrobial

These oils are permitted in accordance with EU regulation (Reg. (EU) 1334/2008) and Codex Alimentarius. In supplements, they are used in the form of capsules, emulsions, drops, or tea blends with added essential oils. For their use in functional foods, it is important to adhere to recommended doses according to EFSA guidelines. For religious certifications (halal/kosher), excipients and manufacturing processes must also be taken into account (EFSA, 2021; FAO/WHO, 2001).

Essential oils represent an important segment of natural dietary supplements due to their functional and aromatic properties. Their safety and usability in the food industry depend on origin, extraction method, purity, and regulatory compliance.

Conclusion

The safety and applicability of essential oils in the food industry depend on origin, extraction method, purity, and regulatory compliance. They are promising ingredients in food supplements, but standardization of analytical, regulatory, and labelling practices is necessary to ensure their safety and efficacy. The standardization process includes: identification of the plant source, GC–MS analysis, establishing limits for active and toxic components, and compliance with purity and stability requirements. For the development, production, and use of essential oils in food supplements, standardization must be the starting and final point of every process. This is the only way to ensure a safe, effective, and legally acceptable product for the end user. Harmonization of ISO and GRAS standards, introduction of GC–MS as a mandatory test, digital verification of origin, and inter-institutional collaboration (EFSA, FDA, ISO) are recommended.

References

Bakkali, F., Averbeck, S., Averbeck, D., Idaomar, M. (2008): Biological effects of essential oils: A review, *Food and Chemical Toxicology* 46(2), 446–475.

Burt, S. (2004): Essential oils: Their antibacterial properties and potential applications in foods—A review, *International Journal of Food Microbiology* 94(3), 223–253.

CFR. (2024): Title 21 Code of Federal Regulations §182.20 – Essential oils as GRAS. U.S. Government Publishing Office. Retrieved August 31, 2025, from https://www.ecfr.gov/current/title-21/section-182.20

Chemat, F., Rombaut, N., Sicaire, A. G., Meullemiestre, A., Fabiano-Tixier, A.-S., Abert-Vian, M. (2020): Essential oils as food additives, *Trends in Food Science Technology* 99, 181–193.

Codex Alimentarius. (2024): General standard for food additives (CODEX STAN 192-1995). FAO/WHO. Retrieved August 31, 2025, from https://www.fao.org/input/download/standards

Codex Alimentarius. (2024): Guidelines for vitamin and mineral food supplements (CAC/GL 55-2005). FAO/WHO. Retrieved August 31, 2025, from https://www.fao.org/input/download/standards

Compliance Gate. (2025): Essential oil regulations in the European Union. Retrieved August 31, 2025, from https://www.compliancegate.com/essential-oil-regulations-european-union/

da Silva Bomfim, N., Meireles, M. A., Mercadante, A. Z. (2020): Essential oils for food preservation: A review, *Food Chemistry* 331, 127002.

Dorff, E. N., Newman, L. E. (Eds.). (1995): Contemporary Jewish ethics and morality. Oxford University Press.

European Commission. (2006): Regulation (EC) No 1925/2006 on the addition of vitamins and minerals and of certain other substances to foods. Retrieved August 30, 2025, from https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32006R1925

European Commission. (2008): Regulation (EC) No 1334/2008 on flavourings and certain food ingredients with flavouring properties for use in and on foods. Retrieved August 30, 2025, from https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32008R1334

FAO. (2023): Good agricultural practices (GAP). Retrieved August 31, 2025, from https://www.fao.org/gap

FAO/WHO. (2001): Codex Alimentarius: Guidelines for the use of flavourings. FAO/WHO Joint Expert Committee on Food Additives.

FAO/WHO. (2023): About Codex Alimentarius. Retrieved August 31, 2025, from https://www.fao.org/fao-who-codexalimentarius

FDA. (2021): Generally recognized as safe (GRAS). U.S. Food and Drug Administration. Retrieved August 31, 2025, from https://www.fda.gov/food/food-ingredients-packaging/generally-recognized-safe-gras

FDA. (2023): Dietary supplements. U.S. Food and Drug Administration. Retrieved August 31, 2025, from https://www.fda.gov/food/dietary-supplements

FDA. (2024): GRAS Substances (SCOGS) Database. Retrieved August 31, 2025, from https://www.fda.gov/food/generally-recognized-safe-gras

General Conference of Seventh-day Adventists. (2012): Nutrition and vegetarianism. Retrieved September 3, 2025, from https://www.adventist.org/health/nutrition/

Gharsallaoui, A., Roudaut, G., Chambin, O., Voilley, A., Saurel, R. (2007): Applications of spray-drying in microencapsulation of food ingredients: An overview, *Food Research International* 40(9), 1107–1121. https://doi.org/10.1016/j.foodres.2007.07.004

Green Source Organics. (2025): Product testing. Retrieved August 31, 2025, from https://www.gsoextracts.com

Halal Control GmbH. (2022): Halal certification standards. Retrieved September 3, 2025, from https://halalcontrol.de/

Harvey, P. (2012): An introduction to Buddhism: Teachings, history and practices (2nd ed.). Cambridge University Press.

Hussaini, M. M., Sakr, A. H. (1984): Islamic dietary laws and practices (2nd ed.). Islamic Food and Nutrition Council of America.

Ibáñez, C. (2016): The sweet smell of standards. Perfumer Flavorist. Retrieved August 31, 2025, from https://www.perfumerflavorist.com/fragrance/regulatory-research/article/21860629

IFOAM. (2022): The IFOAM norms for organic production and processing. IFOAM Organics International.

ISO. (2002): ISO 3515:2002 Oil of lavender (Lavandula angustifolia Mill.). International Organization for Standardization.

ISO. (2013): ISO 9235:2013 Aromatic natural raw materials – Vocabulary. International Organization for Standardization.

ISO. (2024a): ISO/FDIS 3518:2024 Essential oil of sandalwood (Draft standard).

ISO. (2024b): ISO 14714:2024 Determination of benzene in essential oils. Retrieved August 31, 2025, from https://genorma.com

ISO. (2025): ISO/TC 54 – Essential oils. Retrieved August 31, 2025, from https://www.iso.org/committee/48956.html

ISO. (2025a): Committee information: ISO/TC 54 – Essential oils. Retrieved August 31, 2025, from https://www.iso.org/committee/48956.html

ISO. (2025b): Catalogue of ISO standards on essential oils. Retrieved August 31, 2025, from https://www.iso.org/contents/data/committee/04/89/48956/catalogue/

ISO. (2025c): ISO/TS 24106:2021 – Harmonisation of ingredient names. Retrieved August 31, 2025, from https://standards.iteh.ai

Kasper, S., Gastpar, M., Müller, W. E., Volz, H. P. (2010): Efficacy of Silexan in patients with anxiety disorders: A randomized, placebo-controlled trial, *Phytomedicine* 17(2), 94–99.

Orthodox Union. (2023): Kosher certification guidelines. Retrieved September 3, 2025, from https://oukosher.org/

Pavoni, L., Perinelli, D. R., Bonacucina, G., Cespi, M., Palmieri, G. F. (2020): An overview of micro- and nanoemulsions as vehicles for essential oils: Formulation, preparation and stability, *Nanomaterials* 10(1), 135.

Petina, O. (2023): Comparative analysis of the EU regulatory requirements for essential oils (Master's thesis). DGRA.

Ph. Eur. (2023): European Pharmacopoeia (11th ed.). Council of Europe.

Saka, R., Chella, N (2020): Nanotechnology for delivery of natural therapeutic substances: A review, *Environ Chem Lett* 19, 1097–1106.

Singh, N. (2005): Sikhism: A guide for the perplexed. Bloomsbury.

Smith, P. (2008): An introduction to the Baha'i faith. Cambridge University Press.

Szente, L., Szejtli, J. (2004): Cyclodextrins as food ingredients, *Trends in Food Science Technology* 15(3–4), 137–142.

Turek, C., Stintzing, F. C. (2013): Stability of essential oils: A review, *Comprehensive Reviews in Food Science and Food Safety* 12(1), 40–53.

USP. (2023): United States Pharmacopeia 46 – NF 41. United States Pharmacopeial Convention.

Vine, P. (2011): Hinduism and health. Oxford University Press.

World Halal Council. (2020): Global halal standards and certification. WHC Publications.

Hrana u zdravlju i bolesti, znanstveno-stručni časopis za nutricionizam i dijetetiku (2025) 14 (2) 96-102
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ANALIZA NUTRITIVNIH RIZIKA KOD OBOLJELIH OD MULTIPLE SKLEROZE

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Sažetak

Multipla skleroza (MS) je kronična autoimuna, upalna i neurodegenerativna bolest središnjeg živčanog sustava koja zahvaća različite funkcionalne strukture te značajno narušava kvalitetu života oboljelih. Na manifestaciju i progresiju bolesti, uz genetske predispozicije, mogu utjecati čimbenici životnog stila, uključujući prehranu i opći nutritivni status. Cilj ovog rada bio je procijeniti nutritivne rizike kod oboljelih od MS-a uključujući stanje uhranjenosti, rizik od disfagije i prehrambene navike. U istraživanju je sudjelovalo deset ispitanika u dobi od 20 do 67 godina, od kojih je devet bilo ženskog spola. Najviše ih je imalo relapsno-remitentni MS (RRMS), uz trajanje bolesti od 1 do 21 godine. Prema indeksu tjelesne mase, nitko nije bio pothranjen, dok je 2/10 klasificirano kao pretili. Procjena prema EDSS skali pokazala je da polovica bolesnika ima određeni stupanj invaliditeta. Kvaliteta prehrane značajno odstupa od smjernica za MS; 4/10 preskače obroke, rijetko konzumiraju ribu i orašaste plodove. Rizik od disfagije, koja povećava rizik za pothranjenost, prema DYMUS upitniku utvrđen je kod 6/10 ispitanika od kojih jedan ima visok rizik. Rezultati pokazuju da je oboljelima potrebno pružiti ciljanu individualiziranu nutritivnu edukaciju kako bi se podržala neurološka funkcija, imunološki odgovor i postigla bolja kontrola MS-a.

Ključne riječi: multipla skleroza, nutritivni rizici, EDSS skala, DYMUS upitnik

Uvod

Multipla skleroza (MS) je kronični, upalni i neurodegenerativni poremećaj središnjeg živčanog sustava (SŽS), koji pogađa preko 2,8 milijuna ljudi diljem svijeta (Portaccio i sur., 2024). MS je prevalentan neurološki poremećaj koji značajno utječe na javno zdravlje diljem Europe. Multipla skleroza tipično počinje između 20. i 40. godine života, iako do 10 % pacijenata doživi prvu kliničku manifestaciju prije 18. godine, recentni epidemiološki podaci ukazuju na sve veću incidenciju bolesti i u populaciji starijoj od 50 godina (Portaccio i sur. 2024). Na temelju inicijalnog toka bolesti, MS se tradicionalno klasificira kao relapsno-remitentna (RR, 85–90 % pacijenata) ili primarno progresivna (PP, 10–15 %). Većina pacijenata s relapsno-remitentnim oblikom MS-a (RRMS) tijekom vremena razvije sekundarno progresivni tok (SPMS), obilježen postupnim nakupljanjem invalidnosti (Portaccio i sur., 2024).

Tijekom posljednjih desetljeća, bilježi se porast incidencije MS-a (Melcon i sur., 2014), s dominacijom žena (ukupni omjer 3:1) (Portaccio i sur., 2024), a problem se dodatno pogoršao s pandemijom koronavirusa (Korsukewitz i Wiendl, 2024). Iako patološki mehanizmi uključeni u razvoj MS-a još uvijek nisu u potpunosti razjašnjeni, postoje brojni dokazi koji upućuju na to da MS proizlazi iz složene interakcije individualne genetske osjetljivosti i vanjskih okolišnih čimbenika. S obzirom na kratko razdoblje u kojem su se te promjene dogodile, značajan porast incidencije MS-a u populaciji pripisuje se intenzivnim promjenama u okolišu i životnim navikama. Za razliku od genetskih čimbenika rizika, okolišni i životni čimbenici podložni su promjenama, što otvara mogućnosti za prevenciju, osobito u populacijama s povećanim rizikom (Correale i Marrodan, 2022).

Među životne navike ubrajaju se način prehrane, tjelesna aktivnost i psihičko stanje, pri čemu je prehrana prepoznata kao jedan od ključnih čimbenika zdravlja, s posebnim značajem za osobe koje se suočavaju s MS-om (Jurašić i sur., 2018), s naglaskom na vitamin D, omega-3 masne kiseline i antioksidanse za usporavanje progresije bolesti.

Glavni cilj prehrane kod oboljelih od MS-a je uspostavljanje ravnoteže između proupalnih i protuupalnih čimbenika u organizmu (Jurašić i sur., 2018). Pažljivo planiranom prehranom može se utjecati na procese upalnih reakcija koje se javljaju nakon obroka, tijekom procesa razgradnje hrane i njezine apsorpcije, kao i na sistemske učinke nutrijenata iz hrane. Pravilna prehrana može smanjiti

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kronični umor, poboljšati funkciju probavnog trakta, povećati energetske rezerve organizma, poboljšati pokretljivost, prevenirati osteoporozu, a ima i pozitivan učinak na opće emocionalno stanje, kognitivne sposobnosti i mogućnost koncentracije (Jurašić i sur., 2018).

Jedan od simptoma koji dodatno ubrzava progresiju bolesti uzrokujući pothranjenost je disfagija (poteškoće s gutanjem) koja direktno može dovesti do smanjenog unosa hrane i pothranjenosti (Yamout i Alroughani, 2018; NHS 2021; MSSociety, 2021).

S obzirom na važnost prehrane, a u kontekstu pogoršanja kliničke slike MS-a kod narušenog stanja uhranjenosti i nepovoljne prehrane, cilj ovog rada bio je procijeniti status uhranjenosti i rizik od pothranjenosti kod oboljelih te ispitati usklađenost njihovih prehrambenih navika s preporukama za MS.

Ispitanici i metode

Cilj istraživanja

Cilj ovog istraživanja bio je utvrditi status uhranjenosti, procijeniti rizik od pothranjenosti kroz rizik od disfagije i analizirati usklađenost prehrambenih navika oboljelih od MS-a s trenutno važećim preporukama za MS.

Ispitanici

U istraživanje je bilo uključeno 10 osoba (9 žena, 1 muškarac) u dobi od 20 do 67 godina kojima je dijagnosticiran MS. Svi se liječe na Klinici za neurologiju Kliničkog bolničkog centra Osijek i regrutirani su tijekom kolovoza 2021. godine u sklopu svojih redovitih kontrolnih pregleda. Istraživanje je provedeno uz informirani pristanak svih sudionika te u skladu s etičkim načelima. Etičko povjerenstvo Kliničkog bolničkog centra dalo je suglasnost za provedbu ovog istraživanja. Nakon upoznavanja s ciljevima istraživanja, ispitanici su potpisali Suglasnost za sudjelovanje.

Metode

Istraživanje je dizajnirano kao presječno opažajno istraživanje, bez kontrolne skupine. Podaci su prikupljani putem strukturiranog upitnika kreiranog posebno za potrebe ovog rada, koji je obuhvaćao nekoliko ključnih segmenata: opće informacije (dob, spol), kliničke podatke o bolesti (klinički oblik, godina nastanka i trajanje bolesti), antropometrijske mjere, te procjene uhranjenosti i prehrambenih navika.

Podaci o duljini trajanja bolesti, tipu MS-a, imunomodulatornoj terapiji i dužini liječenja te EDSS skor su preuzeti iz kartona pacijenata.

Antropometrijska mjerenja obuhvatila su mjerenje tjelesne visine (u cm), tjelesnu masu (u kg) i opseg struka, pri čemu su visina i masa određene vagom sa stadiometrom (Seca, UK), a opseg struka neelastičnom mjernom vrpcom. Na temelju visine i mase izračunat je indeks tjelesne mase (BMI, engl. Body Mass Index), koji je korišten za klasifikaciju uhranjenosti prema prema stupnju uhranjenosti (CDC, 2024). Stupanj invaliditeta procijenjen je samoprocjenom ispitanika korištenjem prilagođene EDSS skale (engl. Expanded Disability Status Scale).

Drugi i treći dio upitnika je obuhvatio pitanja o prehrambenim navikama. Pitanja su obuhvatila primjenu dodataka prehrani i enteralnih pripravaka, učestalost konzumacije obroka tokom dana tj. imaju li tendenciju preskakanju obroka, uz ispitivanje učestalosti konzumacije pojedine hrane koja je bogata proteinima (poput mesa, ribe i mliječnih proizvoda), ugljikohidratima (tjestenina i kruh) i masnim kiselinama (orašasti plodovi).

Također je napravljena procjena potencijalnih funkcionalnih teškoća povezanih s hranjenjem, posebice disfagiju za što je korišten skraćeni DYMUS upitnik (engl. Dysphagia in Multiple Sclerosis screening questionnaire). Upitnik obuhvaća pitanja o poteškoćama pri gutanju čvrste i tekuće hrane te osjećaju "knedle u grlu". Rezultati su korišteni za kategorizaciju ispitanika prema riziku od disfagije.

Obrada rezultata

Obrada rezultata napravljena je programskim sustavom Statistica (inačica 14.0, StatSoft Inc., SAD), uz odabranu razinu značajnosti od 0,05 i MS MS Office Excel tabličnim alatom (inačica 2016., Microsoft Corp., SAD). Većina rezultata prikazana je deskriptivno a za ostale su zbog malog broja ispitanika korišteni neparametrijski statistički testovi. Podaci su prikazani za svakog ispitanika pojedinačno.

Rezultati i rasprava

U istraživanje je uključeno deset oboljelih od MS-a, devet žena i jedan muškarac u dobi od 20 do 67 godina. Mali uzorak i izražena pretežnost žena predstavljaju ograničenje studije, no odražavaju i rijetkost ove bolesti u populaciji te poznatu veću učestalost MS-a kod žena.

Za bolji uvid u nutritivni status ispitanika, osnovni antropometrijski podaci prikazani su u tablici 1.

Tablica 1. Dob, spol i antropometrijski pokazatelji oboljelih od multiple skleroze

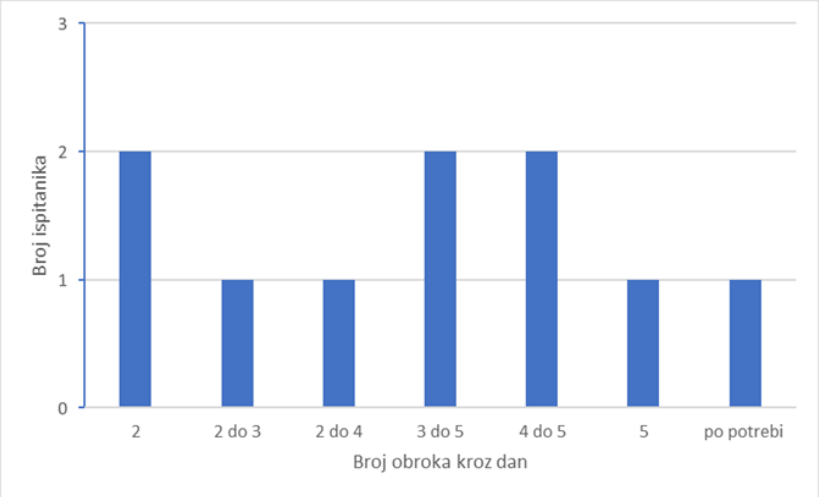
Ispitanik	Spol	Dob (godine)	Visina (cm)	Težina (kg)	Opseg struka (cm)	BMI (kg/m2)	WHtR (omjer struk/visina)
01	M	49	174	65	82	21.5	0.47
02	Ž	53	164	88	110	32.7	0.67
03	Ž	51	158	60	86	24,0	0,54
04	Ž	28	173	65	73	21,7	0,42
05	Ž	43	178	68	70	21,5	0,39
06	Ž	36	158	92	105	36,9	0,67
07	Ž	62	160	60	80	23,4	0,50
08	Ž	20	164	67	81	24,9	0,49
09	Ž	67	163	60	71	22,6	0,43
10	Ž	26	172	68	74	22,9	0,43

Izračunati BMI pokazuje da većina ispitanika spada u kategoriju normalne uhranjenosti, dok je kod dvoje prisutna pretilost (ispitanici 2 i 6), što može biti relevantno za prehrambene i metaboličke preporuke u kontekstu MS-a. Opseg struka i WHtR (engl. waist-to-height ratio) dodatno naglašavaju varijabilnost distribucije tjelesne masnoće među ispitanicima, visoke vrijednosti kod ispitanika 2 i 6 upućuju na povećan abdominalni rizik, što se negativno odražava na njihovo kardiometaboličko zdravlje. Pretilost se u literaturi prepoznaje kao jedan od okolišnih čimbenika rizika za MS, posebno pretilost u ranom djetinjstvu koja povećava vjerojatnost dijagnosticiranja bolesti u kasnijoj dobi (Guerrero-García i sur., 2016). Osim toga, pretilost može negativno utjecati na progresiju MS-a, odgovor na liječenje te doprinosi neuroinflamaciji (Novo i Batista, 2017).

Vrijednosti BMI-a i WHtR ukazuju na potrebu individualiziranog praćenja nutritivnog statusa kod oboljelih od MS-a, jer tjelesna kompozicija može utjecati na opće zdravstveno stanje, funkcionalne sposobnosti i progresiju bolesti. Heterogenost u dobi, spolu i antropometrijskim pokazateljima također naglašava važnost personaliziranog pristupa u kliničkom praćenju i planiranju prehrane.

Analiza kliničkih karakteristika pokazala je da 8/10 ispitanika ima RRMS. Trajanje bolesti varira od nedavno dijagnosticirane do 21 godinu. Imunosupresivi ili druga linija terapije se koristi kod 6/10 ispitanika, dok 2/10 ne prima terapiju zbog nuspojava. Polovica ispitanika ima EDSS skor ≥ 5, što, prema podacima iz izvještaja „Multiple Sclerosis: Current Status and Strategies for the Future“ (Institute of Medicine, US, 2001), odgovara stanju kada je osoba pokretna bez pomagala ili odmora oko 200 m, ali je invaliditet dovoljno težak da spriječi obavljanje svih dnevnih aktivnosti, a skor značajno pozitivno korelira s trenutnom dobi (r=0.729) i dobi pri dijagnozi (r=0.684) što ukazuje na viši stupanj invaliditeta kod starijih i onih s duljim trajanjem bolesti.

Promatrajući prikaz obroka ispitanika kroz dan (Slika 1), vidljivo je da većina ispitanika ne konzumira preporučenih pet obroka dnevno. Samo jedan ispitanik redovito unosi svih pet obroka, dok se kod ostalih broj obroka kreće od 2 do 5 dnevno. Četiri ispitanika (4/10) pokazuju tendenciju preskakanja obroka, što može rezultirati neadekvatnim unosom energije i nutrijenata.



Slika 1. Prikaz broja obroka u danu kod oboljelih od multiple skleroze

U nastavku je promatrana konzumacija ključnih namirnica koje su važne za protuupalni učinak hrane (Tablica 2), što je prethodno istaknuto kao neophodno za dijetoterapiju MS-a kako bi se uspostavila ravnoteža između proupalnih i protuupalnih čimbenika u organizmu (Jurašić i sur., 2018). Prehrana i nutritivni čimbenici utječu na mehanizme patoloških promjena u MS-u, njegov razvoj i stupanj aktivnosti bolesti (Stoiloudis i sur., 2022). Antioksidativni čimbenici u prehrani mogu smanjiti oksidativni stres i pomoći u zaštiti od kronične demijelinizacije te oštećenja neurona ili aksona. Istraživanja upućuju da antioksidativni spojevi poput vitamina D i masnih kiselina mogu doprinijeti regulaciji oksidativnog stresa (Stoiloudis i sur., 2022).

Tablica 2. Učestalost konzumacije promatranih skupina hrane tijekom mjesec dana kod oboljelih od multiple skleroze

	Jogurt ili puding na bazi mlijeka	Jaja (posebno ili kao dio obroka)	Tjestenina	Riba	Orašasti plodovi (kao međuobrok)	Sir ili sirni namaz
Nijednom	1	1	0	1	2	0
1 dan/mjesec	1	1	1	3	1	2
2 - 3 dana/mjesec	1	4	2	2	3	1
1 dan/ tjedan	0	0	2	1	0	1
2 dan/ tjedan	0	3	4	2	0	0
3 dan/tjedan	0	0	0	0	0	2
4 dana/tjedan	2	0	0	1	1	0
5 dana/tjedan	1	1	1	0	1	0
6 dana/tjedan	2	0	0	0	0	2
7 dana/tjedan	2	0	0	0	2	2

Promatrana je konzumacija kruha (kriške kruha na dan, uključujući kruh sa sirom ili sirnim namazom), mlijeka (šalice mlijeka i mliječnih napitaka, uključujući puding na bazi mlijeka), jogurta, mesa, jaja, tjestenine, ribe i orašastih plodova na tjednoj i mjesečnoj bazi. Svakodnevno jogurte i pudinge na bazi mlijeka jede 2/10 ispitanika a nikada 1/10. Mlijeko i mliječni proizvodi su izuzetno važan izvor proteina, ali i kalcija, vitamina D i B12. Istraživanje Muris i sur. (2016) utvrdilo je da je smanjena razina cirkulirajućeg 25-OH D povezana s ranijim prelaskom oboljelih iz faze relapsa i remisije u fazu sekundarne progresije. Primjerice, status vitamina D osoba u fazi sekundarne progresije bio je značajno niži (38 nmol/L) nego u osoba koje nisu ušle u tu fazu (55 nmol/L) (Muris i sur., 2016), te da je niža koncentracija vitamina D u serumu povezana s većim stupnjem invaliditeta EDSS >3 (Bagur i sur., 2017). Također, istraživanja sugeriraju da visoke serumske koncentracije vitamina D mogu smanjiti rizik od novih lezija te poboljšati hod i stanje moždanih lezija. Nalazi sugeriraju da se cirkulirajuće koncentracije vitamina D mogu smatrati biomarkerom MS-a, a dodatni unos vitamina D može se koristiti i terapeutski. Druga istraživanja upućuju na negativnu korelaciju između serumske koncentracije vitamina B12 i EDSS rezultata. Vitamin B12 ima ključnu ulogu u funkciji središnjeg živčanog sustava, osobito u reakciji metionin sintaze koja pretvara homocistein u metionin, što je bitno za sintezu DNA i RNA. Stoga, nedostatak vitamina B12 može dovesti do povećanja koncentracije homocisteina. Potrebna su daljnja istraživanja kako bi se utvrdilo može li suplementacija vitaminom B12 usporiti progresiju MS-a (Bagur i sur., 2017).

Ipak, kod mliječnih proizvoda oboljelima bi trebalo ukazati na činjenicu kako se ne preporučuju punomasni proizvodi, za koje je potvrđen negativan utjecaj kako na rizik tako i progresiju MS-a (Katz Sand, 2018).

Masne kiseline, osobito omega-3 polinezasićene masne kiseline (PUFA), povezane su s ublažavanjem neurodegeneracije kod MS-a. Unos PUFA kroz ribu, orašaste plodove i sjemenke povezan je s zaštitnim učincima protiv demijelinizacije (Katz Sand, 2018). U skladu s literaturom koja naglašava važnost omega-3 PUFA za ublažavanje neurodegeneracije kod MS-a, dobiveni rezultati pokazuju da većina ispitanika (6/10) konzumira ribu minimalno tri puta mjesečno, dok samo dva ispitanika svakodnevno konzumiraju orašaste plodove. S obzirom na to da je unos PUFA kroz ribu i orašaste plodove povezan sa zaštitnim učincima protiv demijelinizacije (Katz Sand, 2018), rezultati upućuju na umjereni unos ovih važnih nutrijenata među ispitanicima, što može imati implikacije za nutritivne preporuke u populaciji oboljelih od MS-a.

Kada se usporedi unos hrane bogate omega-3 masnim kiselinama (npr. riba, orašasti plodovi) ili unos kroz dodatke prehrani, oboljeli se češće odlučuju za suplementaciju. Treba napomenuti kako se upravo vitamin D i omega-3 masne kiseline često prepisuju oboljelima od MS-a uz standardnu terapiu zbog dokazanog pozitivnog učinka na progresiju bolesti

(Tryfonos i sur., 2019). Razlozi zbog kojih se upravo ovi nutrijenti, ali i brojni drugi snažni antioksidansi kao što su vitamin A i polifenoli, preporučuju kod MS-a je djelovanje na jedan od patofizioloških mehanizama odnosno na oksidativni stres (Stoiloudis i sur., 2022).

Posljednjih godina raste interes za vezom između prehrambene suplementacije i MS-a. Fokus je uglavnom na etiologiji i utjecaju dodataka prehrani kao potencijalnog faktora u ublažavanju simptoma. Nekoliko kliničkih studija ispitivalo je učinke masnih kiselina, posebice omega-3 (ω-3) i linolne kiseline, na pacijente s MS-om. Omega-3 masne kiseline (ω-3 FA) imaju ključnu ulogu u normalnoj funkciji mozga i središnjeg živčanog sustava (Tryfonos i sur., 2019).

Unatoč tome što kod niti jednog ispitanika BMI nije ukazivao na pothranjenost, enteralni pripravci su propisani za 2/10 ispitanika. Enteralni pripravci se oboljelima od MS-a prepisuju s ciljem stabilizacije nutritivnog statusa, a kako bi utjecalo na progresiju i kliničku sliku MS-a (Burgos i sur., 2018), a također kada poremećaj gutanja odnosno disfagija postane ozbiljniji, treba razmotriti primjenu enteralne prehrane (Restivo i sur., 2024).

Disfagija je česta i potencijalno životno ugrožavajuća komplikacija MS-a. Poremećaji gutanja mogu se pojaviti u svim fazama MS-a, iako njihova učestalost raste s dobi, trajanjem bolesti i u progresivnim fenotipovima. Specifični upitnici, validirani za disfagiju povezanu s MS-om, poput DYMUS-a, mogu pomoći u identificiranju oboljelih sa subkliničkim poremećajem gutanja ili onih koji su u riziku od razvoja disfagije (Restivo i sur., 2024).

Tablica 3. Rezultati DYMUS skale kod oboljelih od multiple skleroze

Ispitanik	01	02	03	04	05	06	07	08	09	10
Imate li poteškoće prilikom gutanja krute hrane (meso kruh i sl.)?	0	0	1	0	0	0	0	0	0	0
Imate li poteškoće prilikom gutanja tekuće hrane (voda, mlijeko i sl.)?	0	0	0	0	0	0	0	0	0	0
Javlja li Vam se osjećaj knedle u grlu kada gutate hranu/pića ?	0	0	0	0	1	0	0	0	1	0
Lijepi li Vam se hrana u grlu prilikom gutanja?	0	0	0	0	0	0	0	0	0	0
Javlja li Vam se kašalj ili osjećaj gušenja nakon gutanja krute hrane?	0	0	0	0	0	0	0	0	0	0
Javlja li Vam se kašalj ili osjećaj gušenja nakon gutanja tekućina?	0	0	0	0	0	0	0	0	0	0
Morate li nekoliko puta gutati kako bi kruta hrana u potpunosti ″sišla″u želudac?	0	0	1	0	0	0	0	0	0	0
Morate li hranu rezati na male komadiće kako bi ju mogli progutati?	0	0	1	0	0	0	0	1	0	0
Morate li tekućinu uzimati u više gutljaja?	0	0	1	0	0	0	0	0	0	0
Jeste li izgubili na tjelesnoj težini? (u periodu od zadnjih 6 mjeseci)	1	0	0	1	1	0	0	1	1	0
Zbroj	1	0	4	1	2	0	0	2	2	0

Rezultati DYMUS skale prikazani su u tablici 3. Zbrojem pozitivnih odgovora na kraju ljestvice dolazi se do slijedećeg zaključka. Rezultati DYMUS skale pokazuju da 4/10 ispitanika nema problema s gutanjem, dok je preostalih 6 ispitanika u riziku od disfagije. Ispitanici 1 i 4 (skor 1) te 5, 8 i 9 (skor 2) su u riziku, a ispitanik 3 (skor 4) ima alarmantno stanje. Unatoč tome, stanje uhranjenosti promatrano kroz BMI i druga antropometrijska mjerenja (Tablica 1) nije ugroženo. Ispitanici 8 i 9 uzimaju enteralne pripravke, što ih čini nutritivno zbrinutima i smanjuje rizik od pothranjenosti, međutim ispitanik 3 koji ima najviši skor na DYMUS skali ne koristi enteralne pripravke, i kod njega bi trebalo intenzivirati praćenje kako bi se izbjeglo pogoršanje kliničke slike MS-a zbog pothranjenosti.

Zaključak

Na temelju provedenog istraživanja, unatoč malom broju ispitanika, može se zaključiti da unatoč činjenici što antropometrijski podatci ne ukazuju na problem s pothranjenošću već naprotiv pretilošću, rizik od disfagije je prisutan kod polovice oboljelih od MS-a. Prehrambene navike oboljelih od MS-a nisu u skladu s preporukama, odosno učestalo je preskakanje obroka i mali unos hrane bogate nutrijentima koji imaju protuupalno i antioksidacijsko djelovanje. Također, vidljiva su značajna odstupanja s obzirom na dob oboljelih i duljinu trajanja MS-a što uz sve navedeno naglašava potrebu individualnog savjetovanja i redovitog praćenja nutritivnih rizika koji mogu pogoršati kliničku sliku i kvalitetu ivota oboljelih od MS-a.

Literatura

Bagur, M. J., Murcia, M. A., Jiménez-Monreal, A. M., Tur, J. A., Bibiloni, M. M., Alonso, G. L., Martínez-Tomé, M. (2017): Influence of diet in multiple sclerosis: a systematic review, *Advances in Nutrition* 8(3), 463-472.

CDC, Centar za kontrolu i prevenciju bolesti (engl. Centers for Disease Control and Prevention) (2024): BMI categories for adults. Dostupno na: https://www.cdc.gov/bmi/adult-calculator/bmi-categories.html#:~:text=BMI%20categories%20for%20adults%2020%20and%20older,other%20factors%20when%20assessing%20an%20individual's%20health Pristupljeno: 3. prosinca 2025.

Correale, J., Marrodan, M. (2022): Multiple sclerosis and obesity: The role of adipokines, *Frontiers in Immunology* 13, 1038393.

Guerrero-García, J. J., Carrera-Quintanar, L., López-Roa, R. I., Márquez-Aguirre, A. L., Rojas-Mayorquín, A. E., Ortuño-Sahagún, D. (2016): Multiple sclerosis and obesity: Possible roles of adipokines, *Mediators of Inflammation* 2016, 4036232.

Jurašić, M. J., Zavoreo, I., Zadro Matovina, L., Grbić, N., Bašić Kes, V. (2018): Prehrana u liječenju multiple skleroze, *Acta Medica Croatica* 72(3), 409-416.

Katz Sand, I. (2018): The role of diet in multiple sclerosis: mechanistic connections and current evidence, *Current Nutrition Reports* 7(3), 150-160.

Korsukewitz, C., Wiendl, H. (2024): Emerging trends and challenges in multiple sclerosis in Europe: rethinking classification and addressing COVID-19 impact. The Lancet Regional Health–Europe.

MS Society (n.d.): Types of MS. Dostupno na: https://www.mssociety.org.uk/about-ms/types-of-ms. Pristupljeno 2. prosinca 2025.

Muris, A. H., Rolf, L., Broen, K., Hupperts, R., Damoiseaux, J., Smolders, J. (2016): A low vitamin D status at diagnosis is associated with an early conversion to secondary progressive multiple sclerosis, *The Journal of Steroid Biochemistry and Molecular Biology* 164, 254-257.

National Center for Biotechnology Information (n.d.): Dostupno na: https://www.ncbi.nlm.nih.gov/books/NBK222389/. Pristupljeno 3. prosinca 2025.

National Multiple Sclerosis Society (n.d.): Vitamin, mineral and supplements. Dostupno na: https://www.nationalmssociety.org/managing-ms/living-with-ms/diet-exercise-and-healthy-behaviors/diet-nutrition/vitamin-mineral-and-supplements. Pristupljeno 3. prosinca 2025.

NHS (n.d.): Overview–Multiple sclerosis. Dostupno na: https://www.nhs.uk/conditions/multiple-sclerosis/. Pristupljeno 2. prosinca 2025.

Portaccio, E., Magyari, M., Havrdova, E. K., Ruet, A., Brochet, B., Scalfari, A., Amato, M. P. (2024): Multiple sclerosis: emerging epidemiological trends and redefining the clinical course, *The Lancet Regional Health–Europe* 44.

Restivo, D. A., Quartarone, A., Bruschetta, A., Alito, A., Milardi, D., Marchese-Ragona, R., Stampanoni Bassi, M. (2024): Dysphagia in multiple sclerosis: pathophysiology, assessment, and management—an overview, *Frontiers in Neurology* 15, 1514644.

Stoiloudis, P., Kesidou, E., Bakirtzis, C., Sintila, S. A., Konstantinidou, N., Boziki, M., Grigoriadis, N. (2022): The role of diet and interventions on multiple sclerosis: a review, *Nutrients* 14(6), 1150.

Tryfonos, C., Mantzorou, M., Fotiou, D., Vrizas, M., Vadikolias, K., Pavlidou, E., Giaginis, C. (2019): Dietary supplements on controlling multiple sclerosis symptoms and relapses: current clinical evidence and future perspectives, *Medicines* 6(3), 95.

Yamout, I. B., Alroughani, R. (2018): Multiple sclerosis, *Seminars in Neurology* 38(2), 212-225.

Novo, A. M., Batista, S. (2017): Multiple sclerosis: implications of obesity in neuroinflammation, *Advances in Neurobiology* 19, 191-210.

ANALYSIS OF NUTRITIONAL RISKS IN MULITPLE SCLEROSIS PATIENTS

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professional paper

Summary

Multiple sclerosis (MS) is a chronic autoimmune, inflammatory and neurodegenerative disease of the central nervous system that affects various functional structures and significantly impairs the quality of life of patients. The manifestation and progression of the disease, in addition to genetic predisposition, can be influenced by lifestyle factors, including diet and general nutritional status.The aim of this study was to assess nutritional risks in patients with MS including nutritional status, risk of dysphagia, and eating habits.The study involved ten subjects aged 20 to 67, nine of whom were female. Most had relapsing-remitting MS (RRMS), with disease duration ranging from 1 to 21 years. According to Body Mass Index, none were underweight, while 2/10 were classified as obese. Based on the EDSS scale, half of the patients had some degree of disability. Diet quality significantly deviates from the guidelines for MS; 4/10 skip meals, rarely consume fish and nuts. The risk of dysphagia, which increases the risk of malnutrition, according to the DYMUS questionnaire was determined in 6/10 respondents, one of whom is at high risk.The results show that patients need targeted individual nutritional education in order to support neurological function, immune response and achieve better control of MS.

Keywords: multiple sclerosis; nutritional risks; EDSS scale; DYMUS questionnaire

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Books:

Doyle, M.P., Beuchat, L.R., Montville, T.J. (2001): Food Microbiology: Fundamentals and Frontiers, Washington, USA: ASM Press, pp. 572-573.

Chapter in book:

Varoquaux, P., Wiley, R.C. (1994): Biological and Biochemical Changes in Minimally Processed Refrigerated Fruits and Vegetables. In: Minimally Processed Refrigerated Fruits and Vegetables, Wiley, R.C. (ed.), New York, USA: Chapman, pp. 226-268.

Conference proceedings:

Babić, J., Šubarić, D., Ačkar, Đ., Kopjar, M. (2008): Utjecaj hidrokoloida na reološka svojstva voštanog kukuruznog škroba. In: 43rd Croatian and 3rd International Symposium on Agriculture, Pospišil, M. (ed.), Zagreb, HR, pp. 558-562.