

## Assessment of qualitative parameters in fermented plant-based dairy alternatives: a comparative study

BILJANA TRAJKOVSKA – BLANKA TOBOLKOVÁ –  
KRISTÍNA KUKUROVÁ – JANKA KUBINCOVÁ – BOŽENA SKLÁRŠOVÁ

### Summary

This study evaluated commercially available plant-based dairy alternatives: soya, almond, cashew, oat, and coconut, supplied from supermarkets in Bratislava, Slovakia. Physicochemical, textural, nutritional, antioxidant, and colour properties were analysed to characterise the samples. Almond dairy alternative exhibited the highest pH and water-holding capacity (*WHC*), while oat dairy alternative showed the lowest pH but comparable *WHC*. Soya dairy alternative had the highest titratable acidity (*TA*) and lactic acid content, suggesting intense fermentation. Textural analysis revealed superior firmness and consistency in almond and oat dairy alternatives, whereas cashew dairy alternative had the weakest structure. Fatty acid profiling highlighted the nutritional benefits of each base, such as medium-chain fatty acids in coconut and polyunsaturated fatty acids in soya. Cashew and soya dairy alternatives exhibited the highest total phenolic content (*TPC*) and total flavonoid content (*TFC*), which correlated with enhanced antioxidant activity. Colour analysis showed distinct *L\**, *a\**, and *b\** values across samples, with coconut dairy alternative being the lightest. Multivariate analyses successfully discriminated dairy alternatives based on plant origin, with *TA*, *TFC*, fatty acids, and antioxidant parameters being key discriminators. The above findings underscore the potential of plant-based dairy alternatives as functional foods, supporting improved product formulation and targeted consumer marketing.

### Keywords

dairy alternatives; plant-based; quality; fermented

The COVID-19 pandemic catalysed a surge in demand for vegan dairy alternatives, propelling sales beyond pre-pandemic levels and driving market size from 3.10 billion USD ( $3.10 \times 10^9$  USD) in 2023 to over 3.70 billion USD ( $3.70 \times 10^9$  USD) in 2024, with a projected compound annual growth rate (CAGR) of 20.8 % through 2032 [1]. In tandem, Europe witnessed a notable increase in plant-derived protein consumption, with a yearly growth rate of 11 %, fuelling interest in novel plant-based dairy alternatives [2]. Such alternatives offer an economical dairy substitute in developing countries, aiming to replicate the sensory properties of conventional yogurt while supporting long-term storage with

viable lactic acid bacteria [3]. However, the fermentation of plant matrices poses challenges due to the diverse nature of plant proteins and their limited coagulation properties [4]. To address this, researchers explore alternatives such as using exopolysaccharide-producing bacteria as fermentation starters, enhancing texture and functionality [5]. Among legumes, soybean is favoured for its high protein content and quality for fermentation with yogurt cultures, although its beany flavour and allergens remain commercial concerns [6]. Similarly, oats are widely employed for their functional properties, being rich in unsaturated fatty acids and natural antioxidants [7]. Cashews and almonds offer nutritional benefits, with cashews

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**Biljana Trajkovska**, Department of Biotechnology, Faculty of Biotechnical Sciences, University St. Kliment Ohridski, Partizanska, bb, 7000 Bitola, North Macedonia.

**Blanka Tobolková, Kristína Kukurová, Janka Kubincová, Božena Skláršová**, Division of Food Chemistry and Analysis, Department of Food Science, National Agricultural and Food Centre, Priemysel'ná 4, 82108 Bratislava, Slovakia.

*Corresponding author:*

Biljana Trajkovska, e-mail: [biljana.trajkovska@uklo.edu.mk](mailto:biljana.trajkovska@uklo.edu.mk)

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containing allergenic proteins and almonds recognised for their functional properties and probiotic stimulation [8, 9]. Coconuts, with their nutritional and medicinal properties, are also commonly used in the production of plant-based dairy alternatives [10].

Despite the increasing popularity of these alternatives, limited information exists on their quality characteristics, prompting our study to evaluate the physicochemical properties, rheological properties, organic acid contents, antioxidant activity and fatty acid composition of commercial soya, almond, cashew, oat, and coconut dairy alternatives in the Slovak market. This assessment seeks to discern variations in technological properties and quality attributes among these products.

## MATERIALS AND METHODS

### Plant-based dairy alternatives

This study analysed five commercially available plant-based dairy alternatives, sourced from local supermarkets in Bratislava, Slovakia (Tab. 1). Plant-based dairy alternatives, made from soya, almond, cashew, oat, and coconut, varied in weight from 120 g to 160 g. Detailed information regarding the nutritional composition and ingredients of each product can be found in Tab. 1 and Tab. 2, respectively.

### Physicochemical characteristics

The pH of the samples was determined at 20 °C using a HI 2211 pH meter equipped with a glass electrode (Hanna Instruments, Woonsocket,

Rhode Island, USA), previously calibrated using standard buffer solutions with pH values of 4.0 and 7.0.

Titrate acidity (*TA*) was assessed following GRASSO et al. [11]. Ten grams of plant-based dairy alternatives were titrated with 0.1 mol·l<sup>-1</sup> NaOH using phenolphthalein as an indicator. Triplicate analyses were conducted, and *TA* was reported as millilitres of NaOH per kilogram of sample.

To measure water holding capacity (*WHC*), 20 g samples were placed in 50 ml tubes and centrifuged at 640 ×g for 20 min at 4 °C using a Sigma 2-16KC centrifuge (Sigma-Aldrich, St. Louis, Missouri, USA). The supernatant was collected after centrifugation and weighed. *WHC* was calculated using the formula described by GRASSO et al. [11], expressed as a percentage.

Water activity (*a<sub>w</sub>*) was determined using a LabMaster *a<sub>w</sub>*-meter (Novasina, Lachen, Switzerland).

### Texture characteristics determination

To assess the textural properties of plant-based dairy alternatives, the back extrusion test was conducted using a TA.XT plus texture analyser (Stable Micro Systems, Godalming, United Kingdom) with the Forward Extrusion Rig (HDP/FE). Samples were stored overnight at 4 °C. Standardised 100 g samples were filled into Perspex polycarbonate cylinder containers and compressed at a test speed of 1.0 mm·s<sup>-1</sup> using a 30 kg load cell at 22 ± 2 °C [11, 12]. Textural parameters were analysed using Exponent software (Stable Micro Systems). Firmness was defined as the maximum positive force required for extrusion (expressed

Tab. 1. Composition of plant-based dairy alternatives.

Product	Country of origin	Components		Other components
		Characteristics	Proportion [%]	
Coconut	Slovakia	Coconut milk (water, coconut extract)	95.6	Starch, coagulant (carob gum), vegan yogurt cultures ( <i>L. delbrueckii</i> subsp. <i>bulgaricus</i> , <i>S. thermophilus</i> )
Soya	Slovakia	Soya base (drinking water, hulled soya beans)	10.7	Sugar, tricalcium citrate, stabiliser (pectins), acidity regulators (sodium citrates, citric acid), flavoring, sea salt, antioxidants (tocopherol-rich extract, fatty acid esters of ascorbic acid), vitamins (B12, D2), yogurt cultures ( <i>S. thermophilus</i> , <i>L. bulgaricus</i> )
Almond	Slovakia	Drinking water, almond	6.8	Modified starch, sugar, tricalcium phosphate, vitamins (B12, D2), natural flavouring, yogurt cultures ( <i>S. thermophilus</i> , <i>L. bulgaricus</i> )
Cashew	Germany	Cashew nut base (drinking water, cashew nuts)	14.0	Yogurt cultures, starch, stabiliser (pectin), sea salt
Oat	Germany	Oat base (drinking water, wholegrain oat)	11.0	Yogurt cultures, coconut fat, starch, coagulant (carob flour), sea salt

**Tab. 2.** Nutritional parameters of plant-based dairy alternatives.

Parameters	Coconut	Soya	Almond	Cashew	Oat
Energy [kcal]	93	51	64	89	83
Energy [kJ]	384	212	266	368	347
Fat [g]	7.1	2.3	3.3	6.6	4.5
of which saturated fat [g]	6.8	0.4	0.3	1.3	3.7
Saccharide [g]	6.4	2.1	6.3	5.2	9.8
of which sugar [g]	0.9	2.1	2	0.5	5.8
Fibre [g]	< 0.5	1	0.3	0.5	0.2
Protein [g]	0.7	4	1.5	2.1	0.7
Salt [g]	0.03	0.25	0.020	0.03	0.10
Lactose [g]	–	0.0	0.0	–	–
Vitamin D [ $\mu$ g]	–	0.75 *	0.75 *	–	–
Vitamin B12 [ $\mu$ g]	–	0.38 *	0.38 *	–	–
Calcium [ $\mu$ g]	–	120 *	140 **	–	–

Value 0.0 indicates a declared content of zero or below the labelling threshold.

(–) – no declaration on the product label, \* – 15 % of reference intake; \*\* – 18 % of reference intake.

in grams), consistency as the area of the positive region (expressed in kilograms per second), stickiness as the force needed to unstick the probe (expressed in kilograms), and adhesiveness as the total work required to unstick the probe (expressed in kilograms per second).

#### Fatty acid methyl esters determination

Fatty acid methyl ester analysis was performed according to TRAJKOVSKA et al. [12], with selected supplementary details provided here for reproducibility. A 100  $\mu$ l volume of each homogenised sample was extracted using methanol (1 ml) and hexane (2 ml), followed by vortex mixing and ultrasonication in an ultrasonic bath (Kraintek, Podhájska, Slovakia). After mixing at 225  $\times$ g for 25 min on a Promax 2020 shaker (Heidolph Instruments, Schwabach, Germany), samples were centrifuged, and 1 ml of the upper fraction was mixed with 100  $\mu$ l of 0.5 mol·l<sup>-1</sup> sodium methoxide and vortexed. Then, 60  $\mu$ l of 0.03 mol·l<sup>-1</sup> oxalic acid was added, and samples were stored at –18 °C for up to one month.

Analysis was performed using gas chromatography-mass spectrometry (GC-MS) on an Agilent 5975C system (Agilent Technologies, Santa Clara, California, USA) equipped with a DB-23 column (60 m  $\times$  250  $\mu$ m  $\times$  0.25  $\mu$ m; J&W Scientific, Folsom, California, USA) using helium as the carrier gas (flow rate of 1.85 ml·min<sup>-1</sup>). The injector temperature was maintained at 230 °C. A 1  $\mu$ l sample volume was injected in split mode with a split ratio of 1:10. Electron ionisation (EI) at 70 eV was used for mass spectrometric detection, and the transfer line temperature was maintained at 250 °C. The oven temperature

programme started at 50 °C (held for 1 min), increased to 100 °C at 25 °C·min<sup>-1</sup>, rose to 175 °C at 4 °C·min<sup>-1</sup> (no hold), and finally advanced to 230 °C with a 5.25 min final hold. Fatty acid methyl esters were identified by comparison of retention times with reference standards (Supelco 37 Component FAME Mix, Sigma-Aldrich), and identification was further confirmed by comparison of mass spectra with the NIST Mass Spectral Library (National Institute of Standards and Technology, Gaithersburg, Maryland, USA). Quantification was performed semi-quantitatively based on peak area normalisation, without the use of an internal standard. The results were expressed as a relative percentage of total fatty acids.

#### Organic acids determination

Organic acids were extracted using a 95:5 (v/v) mixture of water and methanol. Following the method described by TRAJKOVSKA et al. [12], a 1  $\pm$  0.01 g sample was combined with 10 ml of the extraction mixture, along with 100  $\mu$ l each of Carrez solutions I (prepared by dissolving 15 g of potassium hexacyanoferrate(II) trihydrate in 100 ml of water) and Carrez solution II (prepared by dissolving 30 g of zinc sulphate heptahydrate in 100 ml of water). After shaking, sonication, and centrifugation, the supernatant was filtered through a 0.45  $\mu$ m nylon membrane syringe filter (Frisenette, Knebel, Denmark). Analysis was performed using an Agilent 1290 HPLC system (Agilent Technologies) equipped with a binary gradient pump, an autosampler, and a photodiode array detector (DAD) set at 210 nm. Separation was performed on a Synergi HYDRO-RP column (250 mm  $\times$  4.6 mm, particle size 4  $\mu$ m; Pheno-

menex, Torrance, California, USA) with a mobile phase gradient of water and H<sub>3</sub>PO<sub>4</sub> (99:1, v/v) and acetonitrile. A 10 µl sample injection volume was used, and external calibration was employed to quantify individual organic acids.

#### UV-Vis and EPR measurements

UV-Vis experiments were conducted using a Shimadzu 3600 spectrophotometer (Shimadzu, Kyoto, Japan) with accessories, following TOBOLKOVÁ et al. [13]. For electron paramagnetic resonance (EPR) spectroscopy, a portable X-band EPR spectrometer e-scan (Bruker Biospin, Ettlingen, Germany) was utilised.

#### Extract preparation

Approximately 20 g of the sample was centrifuged at 10 000 ×g for 10 min at 4 °C, and the supernatant was used for evaluating total polyphenol concentration (TPC), total flavonoid concentration (TFC), and antioxidant activity.

#### Total polyphenol determination

100 µl of supernatant was mixed with 7.9 ml of deionised water and 500 µl of Folin-Ciocalteu reagent (Sigma-Aldrich), following the procedure outlined by Trajkovska et al. [12]. After 10 min, 1.5 ml of 200 g·l<sup>-1</sup> sodium carbonate was added, and the mixture was allowed to stand for 60 min at room temperature (22 ± 2 °C). Absorbance was measured at 765 nm. Results were expressed as milligrams of gallic acid equivalent (GAE) per litre of dairy alternative.

#### Total flavonoid determination

1 ml of supernatant was mixed with 2 ml of deionised water following the procedure outlined by TRAJKOVSKA et al. [12]. Then, 150 µl of 50 g·l<sup>-1</sup> sodium nitrite was added. After 6 min, 150 µl of 100 g·l<sup>-1</sup> aluminium chloride hexahydrate was included. Following another 6 min, 2 ml of 40 g·l<sup>-1</sup> sodium hydroxide was added, and after 15 min, absorbance was measured at 510 nm. Results were expressed as milligrams of rutin equivalent (RE) per litre of dairy alternative.

#### Antioxidant activity determination

Exactly 300 µl of sample was mixed with either 700 µl of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS<sup>•+</sup>) in deionised water (initial concentration 0.1 mmol·l<sup>-1</sup>) or 2,2-diphenyl-1-picrylhydrazyl (•DPPH) in ethanol (initial concentration 0.1 mmol·l<sup>-1</sup>). The mixture was purged with 2 ml of air and transferred into an EPR flat cell. EPR measurements started 3 min after the •DPPH or ABTS<sup>•+</sup> addition, recording 10 EPR

spectra in the time domain over 15 min. Each EPR spectrum represented an average of 30 individual scans. The experiments were conducted in duplicates. Results of both assays were expressed as Trolox equivalent antioxidant capacity according to POLOVKA [14].

#### Colour measurement

The colour of each sample was assessed using a UV-3600 double-beam spectrometer (Shimadzu) in a 1 cm cuvette 100-QS-Suprasil (Hellma, Müllheim, Germany), following the method by TOBOLKOVÁ et al. [13]. The setup included a spectral range from 380 nm to 780 nm, with a sampling interval of 2 nm and a slit width of 0.1 nm. Colour values in the CIE *L\*a\*b\** colour system were determined using ColorLite Panorama Shimadzu software v3.1.16 (LabCognition, Shimadzu), employing Illuminant D65 and a 10° standard observer angle. The whiteness index (*WI*) and yellowness index (*YI*) was calculated based on the method by HIRSCHLER [15].

#### Statistical analysis

All analyses were performed using XLSTAT (Lumivero, Paris, France). Data were expressed as mean ± standard deviation (*SD*) based on three independent replicates. Differences among sample groups were determined using one-way analysis of variance (ANOVA), followed by Fisher's least significant difference (*LSD*) test at a 95% confidence interval (*p* < 0.05) to assess statistically significant differences. In addition, multivariate statistical analyses, including principal component analysis (PCA), principal component factoring (PCF), and stepwise discriminant analysis, were conducted using Unistat 6.0 (Unistat, London, United Kingdom) statistical software.

## RESULTS AND DISCUSSION

#### Physicochemical characteristics

The quality of fermented dairy products is influenced by various factors, including starter cultures, milk composition, temperature, pH, homogenisation, stirring, prebiotics and probiotics, and packaging [16]. Tab. 3 presents the physicochemical characteristics of the analysed samples.

The almond dairy alternative stands out among the samples with the highest pH (4.75), indicating that it is the least acidic. Combined with its exceptional *WHC* of 100 %, this suggests that it has a smooth and creamy texture with minimal syneresis. Conversely, the oat dairy alternative is the most acidic, with the lowest pH (4.27) and

**Tab. 3.** Physico-chemical characteristics of plant-based dairy alternatives.

Parameter	Coconut	Soya	Almond	Cashew	Oat
pH	4.66 ± 0.00 <sup>a</sup>	4.63 ± 0.01 <sup>b</sup>	4.75 ± 0.00 <sup>c</sup>	4.61 ± 0.01 <sup>d</sup>	4.27 ± 0.01 <sup>e</sup>
Water activity	0.97 ± 0.00 <sup>a</sup>	0.97 ± 0.00 <sup>a</sup>	0.97 ± 0.01 <sup>a</sup>	0.97 ± 0.01 <sup>a</sup>	0.96 ± 0.00 <sup>b</sup>
Water-holding capacity [%]	97.9 ± 0.4 <sup>b</sup>	97.2 ± 0.1 <sup>c</sup>	100.0 ± 0.0 <sup>a</sup>	79.1 ± 0.2 <sup>d</sup>	99.9 ± 0.1 <sup>a</sup>
Titrateable acidity [ml·kg <sup>-1</sup> ]	420 ± 0 <sup>a</sup>	1715 ± 7 <sup>b</sup>	525 ± 7 <sup>c</sup>	785 ± 7 <sup>d</sup>	305 ± 7 <sup>e</sup>

Values represent mean ± standard deviation ( $n = 3$ ). Standard deviation equal to 0.00 indicate no observed variation among replicates values.

Different small letters in superscript in the same row indicate statistically significant differences ( $p < 0.05$ ).

*TA* of 305 ml·kg<sup>-1</sup>. Despite its higher acidity, it achieves a high *WHC* (99.9 %), indicating it can still retain water effectively, resulting in a cohesive texture. The soya dairy alternative displays a very high *TA* of 1715 ml·kg<sup>-1</sup>, the highest among the samples, but only a moderately acidic pH, similar to the other samples. This discrepancy is likely due to the strong buffering capacity from the presence of acidity regulators and other declared compounds (Tab. 1). The coconut dairy alternative demonstrates a strong *WHC* of 97.9 %, coupled with a moderate pH (4.66) and *TA* (420 ml·kg<sup>-1</sup>). Finally, the cashew dairy alternative shows the lowest *WHC* (79.1 %), indicating a higher likelihood of syneresis, which might affect its appearance and texture. Despite this, its pH (4.61) and *TA* (785 ml·kg<sup>-1</sup>) reflect moderate acidity, providing a balanced flavour profile. Similar results have been reported by other authors [11, 17, 18].

#### Texture characteristics

The textural analyses of the plant-based dairy alternatives (coconut, soya, almond, cashew, and oat) are presented in Tab. 4.

The almond dairy alternative stands out with the highest firmness (622.80 g) and consistency (11.98 kg·s<sup>-1</sup>), making it the thickest and most stable among the samples. This aligns with its excellent *WHC* (100 %), which prevents syneresis and supports a dense, creamy texture. The oat dairy alternative also demonstrates strong textural properties, with high firmness (439.40 g) and consistency (8.50 kg·s<sup>-1</sup>). Its *WHC* (99.9 %) is nearly

that of the almond dairy alternative, contributing to its thick and cohesive structure. The coconut dairy alternative strikes a balance between firmness (393.96 g) and consistency (7.44 kg·s<sup>-1</sup>), supported by a robust *WHC* (97.9 %). In contrast, the soya and cashew dairy alternatives exhibit weaker textural properties. The soya dairy alternative has relatively low firmness (168.48 g) and consistency (3.18 kg·s<sup>-1</sup>), which correlates with its slightly reduced *WHC* (97.2 %). Its minimal stickiness (-0.10 kg) and adhesiveness (-0.11 kg·s<sup>-1</sup>) suggest a smoother but less cohesive texture, which might appeal to those preferring lighter dairy alternatives. The cashew dairy alternative has the lowest firmness (121.97 g) and consistency (2.27 kg·s<sup>-1</sup>), reflecting its poor *WHC* (79.1 %). These characteristics result in a weaker structure prone to syneresis, which could limit its sensory appeal. Its low stickiness (-0.11 kg) and higher adhesiveness (-0.16 kg·s<sup>-1</sup>) further indicate a less stable texture compared to the other samples.

The firmness of plant-based dairy alternatives is influenced by the type of fat, the protein ratio, and the specific lactic acid bacteria (LAB) cultures used. The texture is largely determined by the interactions between proteins, lipids, and stabilisers, which can vary considerably depending on the plant source [19].

#### Fatty acids

The fatty acid profiles of the analysed vegan yogurts (Tab. 5) reflected the characteristic lipid composition of the plant raw materials used in

**Tab. 4.** Textural characteristics of plant-based dairy alternatives.

Parameter	Coconut	Soya	Almond	Cashew	Oat
Firmness [g]	393.96 ± 21.80 <sup>b</sup>	168.48 ± 41.02 <sup>c</sup>	622.80 ± 42.74 <sup>a</sup>	121.97 ± 8.37 <sup>c</sup>	439.40 ± 40.54 <sup>b</sup>
Consistency [kg·s <sup>-1</sup> ]	7.44 ± 0.54 <sup>b</sup>	3.18 ± 0.74 <sup>c</sup>	11.98 ± 0.70 <sup>a</sup>	2.27 ± 0.16 <sup>c</sup>	8.50 ± 0.75 <sup>b</sup>
Stickiness [kg]	-0.13 ± 0.03 <sup>b</sup>	-0.10 ± 0.02 <sup>a</sup>	-0.20 ± 0.02 <sup>b</sup>	-0.11 ± 0.01 <sup>a</sup>	-0.11 ± 0.02 <sup>a</sup>
Adhesiveness [kg·s <sup>-1</sup> ]	-0.03 ± 0.03 <sup>a</sup>	-0.11 ± 0.11 <sup>a</sup>	-0.10 ± 0.10 <sup>a</sup>	-0.16 ± 0.03 <sup>a</sup>	-0.17 ± 0.05 <sup>a</sup>

Values represent mean ± standard deviation ( $n = 3$ ). Different small letters in superscript in the same row indicate statistically significant differences ( $p < 0.05$ ).

their formulation. Individual fatty acids differed in carbon chain length, degree of unsaturation, and their relative proportions within the lipid profile. The results are expressed as relative proportions of fatty acids in the total fatty acid profile (equivalent to weight percent).

The coconut-based alternative was characterised by a predominance of medium-chain saturated fatty acids (MCFAs), particularly lauric acid (C12:0;  $\approx 49.9\%$ ), followed by myristic acid (C14:0;  $\approx 14.7\%$ ) and caprylic acid (C8:0;  $\approx 13.2\%$ ), which corresponds to the known composition of coconut fat. This observation is consistent with previous studies reporting that coconut oil is rich in MCFAs, with lauric acid accounting for approximately 45–50 % of total fatty acids [20]. MCFAs are rapidly metabolised and represent a significant source of energy, and have also been associated with metabolic and cognitive effects [21]. The overall fatty acid profile was characterised by a high proportion of saturated fatty acids (SFAs) and a low content of unsaturated fatty acids (UFAs), mainly represented by oleic acid (C18:1) and linoleic acid (C18:2), while  $\alpha$ -linolenic acid (C18:3) was not detected. Accordingly, the UFA/SFA ( $\approx 0.08$ ) and polyunsaturated fatty acids PUFA/SFA ( $\approx 0.02$ ) ratios were lower than recommended values ( $> 1$  and  $\geq 0.04$ , respectively), and no favourable  $n-6/n-3$  ratio was observed [22–24].

In contrast, the soya-based alternative exhibited a high proportion of unsaturated fatty acids, predominantly linoleic acid (C18:2;  $\approx 41.9\%$ ) and oleic acid (C18:1;  $\approx 24.9\%$ ). The presence of  $\alpha$ -linolenic acid (C18:3;  $\approx 6.2\%$ ) indicates the

occurrence of  $n-3$  fatty acids, contributing to the nutritional quality of soya lipids [25].

Nut-based alternatives (almond and cashew) were characterised by lipid profiles dominated by UFAs, as is typical for nuts and oil-rich seeds. The almond-based alternative showed a high proportion of monounsaturated fatty acids, particularly oleic acid (C18:1;  $\approx 60.4\%$ ), while SFAs were present at lower levels, mainly as palmitic acid (C16:0;  $\approx 10.8\%$ ). These findings are consistent with previously reported fatty acid compositions of almonds [26]. The cashew-based alternative exhibited a similar pattern, with oleic acid (C18:1;  $\approx 49.9\%$ ) as the dominant fatty acid, accompanied by linoleic acid (C18:2;  $\approx 21.1\%$ ). SFAs, primarily palmitic and stearic acids, were present in slightly higher proportions compared to the almond-based alternative.

The oat-based alternative, although primarily derived from oats, was modified by the addition of coconut fat to achieve the desired product texture. This was reflected in the fatty acid profile, particularly in the increased proportions of lauric acid (C12:0;  $\approx 39.8\%$ ) and myristic acid (C14:0;  $\approx 14.4\%$ ). At the same time, oleic acid (C18:1;  $\approx 11.6\%$ ) and linoleic acid (C18:2;  $\approx 6.7\%$ ), originating from the oat fraction, were also present. Consequently, the resulting lipid profile reflects both the raw material composition and formulation practices, with a higher proportion of saturated fatty acids.

Overall, substantial differences in fatty acid composition were observed among the plant-based alternatives, reflecting both the intrinsic properties of the raw materials and product formulation.

**Tab. 5.** Relative proportions of fatty acids in plant-based dairy alternatives.

Fatty acid [%]		Common name	Coconut	Soya	Almond	Cashew	Oat
Hexanoic acid	C6:0	Caproic	0.8 $\pm$ 0.0	nd	nd	nd	0.4 $\pm$ 0.0
Octanoic acid	C8:0	Caprylic	13.2 $\pm$ 0.6	0.2 $\pm$ 0.0	nd	0.2 $\pm$ 0.1	9.4 $\pm$ 0.4
Decanoic acid	C10:0	Capric	7.3 $\pm$ 0.2	0.3 $\pm$ 0.0	0.2 $\pm$ 0.0	0.5 $\pm$ 0.1	5.4 $\pm$ 0.1
Dodecanoic acid	C12:0	Lauric	49.9 $\pm$ 1.1	2.6 $\pm$ 0.1	1.4 $\pm$ 0.1	2.5 $\pm$ 0.2	39.8 $\pm$ 1.9
Tetradecanoic acid	C14:0	Myristic	14.7 $\pm$ 0.4	1.3 $\pm$ 0.0	1.0 $\pm$ 0.1	1.4 $\pm$ 0.1	14.4 $\pm$ 0.8
Hexadecanoic acid	C16:0	Palmitic	5.7 $\pm$ 0.2	13.4 $\pm$ 0.2	10.8 $\pm$ 0.2	10.9 $\pm$ 0.6	9.7 $\pm$ 0.2
9-Hexadecenoic acid	C16:1	Palmitoleic	0.4 $\pm$ 0.1	0.3 $\pm$ 0.1	3.5 $\pm$ 0.1	0.8 $\pm$ 0.2	0.1 $\pm$ 0.1
Octadecanoic acid	C18:0	Stearic	1.4 $\pm$ 0.2	4.9 $\pm$ 0.1	2.1 $\pm$ 0.1	6.1 $\pm$ 0.4	1.7 $\pm$ 0.3
9-Octadecenoic acid, <i>cis</i>	C18:1	Oleic	5.1 $\pm$ 0.9	24.9 $\pm$ 0.3	60.4 $\pm$ 0.4	49.9 $\pm$ 1.5	11.6 $\pm$ 1.1
9-Octadecenoic acid, <i>trans</i>	C18:1	Elaidic	0.1 $\pm$ 0.0	1.5 $\pm$ 0.0	1.0 $\pm$ 0.1	0.4 $\pm$ 0.0	0.1 $\pm$ 0.0
9,12-Octadecadienoic acid	C18:2	Linoleic	1.6 $\pm$ 0.6	41.9 $\pm$ 0.3	19.2 $\pm$ 0.1	21.1 $\pm$ 0.6	6.7 $\pm$ 1.0
9,12,15-Octadecatrienoic acid	C18:3	$\alpha$ -Linolenic	nd	6.2 $\pm$ 0.1	0.4 $\pm$ 0.0	0.8 $\pm$ 0.0	0.2 $\pm$ 0.1
Eicosanoic acid	C20:0	Arachidic	nd	0.4 $\pm$ 0.0	nd	0.4 $\pm$ 0.0	nd

The values represent the relative percentage of total fatty acids. nd – not detected.

From a nutritional perspective, soya- and almond-based alternatives exhibited more favourable fatty acid profiles, whereas coconut-based products were characterised by a predominance of SFAs.

### Organic acids

The Tab. 6 presents the content of organic acids (malic, lactic, acetic, and citric) in plant-based dairy alternatives. Lactic acid was present in all of the samples; the soya-based dairy alternative had the highest level ( $4.06 \text{ g}\cdot\text{kg}^{-1}$ ) compared with coconut and almond, which showed relatively moderate levels (approximately  $2.54 \text{ g}\cdot\text{kg}^{-1}$  and  $2.06 \text{ g}\cdot\text{kg}^{-1}$ , respectively). Lactic acid is produced by fermentation (via lactic acid bacteria), and its content correlated with the active dairy alternative cultures previously used for fermentation, such as *Streptococcus thermophilus*, *Lactobacillus bulgaricus* (Tab. 1). Similar findings were reported by PART et al. [17], with lactic acid being the dominant acid in the plant-based dairy alternatives, ranging from  $3.5 \text{ g}\cdot\text{l}^{-1}$  to  $5.8 \text{ g}\cdot\text{l}^{-1}$ . Citric acid was detected in all samples, with the soya-based dairy alternatives having the highest content ( $5.87 \text{ g}\cdot\text{kg}^{-1}$ ), which originates from added acidity regulators (sodium citrates, citric acid) in the formulation (Tab. 1). Coconut, almond, cashew and oat plant-based dairy alternatives had much lower levels, indicating limited citric acid addition, which was not specified in the formulations. The presence of lactic acid and the absence of acetic acid indicated active homolactic fermentation by *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. Changes in organic acid profiles over time may reflect microbial activity, degradation, or ingredient [27].

### Antioxidant characteristics

Plant-based materials such as soya, oats, and nuts are rich in polyphenols and flavonoids, making them suitable for producing functional food products.

The cashew dairy alternative showed the highest *TPC* ( $194.38 \text{ mg}\cdot\text{l}^{-1}$ ), closely followed by the soya dairy alternative ( $182.60 \text{ mg}\cdot\text{l}^{-1}$ ) (Fig. 1A). This can be attributed to the rich phenolic content of cashew nuts, including caffeic acid, *p*-coumaric acid, ferulic acid, gallic acid, syringic acid, naringenin, catechin, and quercetin [28], and soybeans, which are well-known sources of isoflavones and other phenolics. The oat dairy alternative demonstrated moderate *TPC* ( $123.41 \text{ mg}\cdot\text{l}^{-1}$ ), likely due to the presence of avenanthramides, unique polyphenols found in oats. These compounds are reported to have anti-inflammatory and antioxidant effects [29]. The almond dairy alternative, with a *TPC* of  $104.63 \text{ mg}\cdot\text{l}^{-1}$ , also provided considerable phenolic compounds like flavonoids and phenolic acids, although at lower levels than the cashew and soya plant-based dairy alternatives. The coconut plant-based dairy alternative exhibited the lowest *TPC* ( $58.06 \text{ mg}\cdot\text{l}^{-1}$ ), reflecting the limited phenolic profile of coconut compared to other plant-based materials. Furthermore, variations in *TPC* values can arise due to differences in the raw material composition and the specific production processes employed, up to  $91.18 \text{ mg}\cdot\text{l}^{-1}$  [12].

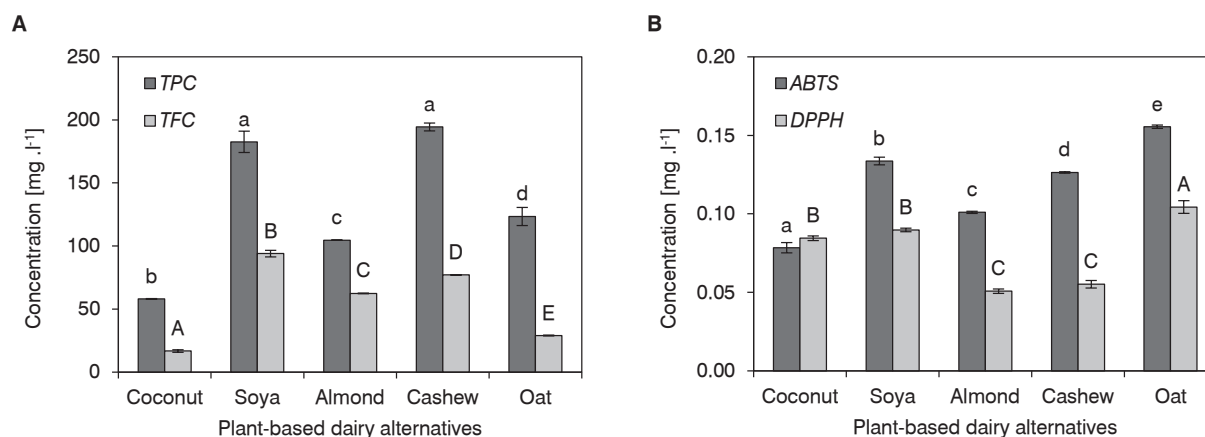
A similar trend was observed in *TFC* values, with cashew dairy alternative ( $77.06 \text{ mg}\cdot\text{l}^{-1}$ ) and soya dairy alternative ( $93.97 \text{ mg}\cdot\text{l}^{-1}$ ) leading, indicating their superior flavonoid content. Flavonoids, such as catechins in cashews and isoflavones in soya, are well documented for their ability to neutralise free radicals, enhance immune function, and promote cardiovascular health. The presence of polyphenols and flavonoids suggests potential functional benefits [30]. Almond dairy alternative also displayed a significant *TFC* ( $62.44 \text{ mg}\cdot\text{l}^{-1}$ ), which complements its moderate *TPC* and indicates its potential for functional health benefits. Oat dairy alternative ( $29.06 \text{ mg}\cdot\text{l}^{-1}$ ) had a lower *TFC* than cashew, soya, and almond dairy alternatives, possibly due to the lower flavonoid levels in oats or flavonoid losses during processing.

**Tab. 6.** Content of organic acids in plant-based dairy alternatives.

	Coconut	Soya	Almond	Cashew	Oat
<b>Organic acid [<math>\text{g}\cdot\text{kg}^{-1}</math>]</b>					
Malic acid	$\leq \text{LOD}$	$\leq \text{LOD}$	nd	$\leq \text{LOD}$	$\leq \text{LOD}$
Lactic acid	$2.54 \pm 0.00$	$4.06 \pm 0.01$	$2.06 \pm 0.01$	$2.18 \pm 0.01$	$0.79 \pm 0.00$
Acetic acid	$\leq \text{LOD}$	nd	$\leq \text{LOD}$	$\leq \text{LOD}$	nd
Citric acid	$0.17 \pm 0.00$	$5.87 \pm 0.01$	$0.23 \pm 0.00$	$0.45 \pm 0.00$	$0.12 \pm 0.00$

Standard deviations equal to 0.00 indicate no observed variation among replicate values.

*LOD* – limit of detection (indicates the presence of a signal at or below the limit of detection), nd – not detected (indicates no detectable signal).



**Fig. 1.** Antioxidant characteristics of the supernatant of plant-based dairy alternatives.

A – total polyphenols and total flavonoids concentration, B – antioxidant activity

*TPC* – total polyphenols concentration (expressed as milligrams of gallic acid equivalent), *TFC* – total flavonoids concentration (expressed as milligrams of rutin equivalent), *AA* – antioxidant activity (expressed as Trolox equivalents), *ABTS* – antioxidant activity determined by the ABTS assay, *DPPH* – antioxidant activity determined by the DPPH assay.

Different lowercase letters (a, b, c, d) indicate significant differences among *TPC* values (part A), and *ABTS* (part B), whereas different uppercase letters (A, B, C, D, E) denote significant differences among *TFC* values (part A), and *DPPH* (part B). Groups sharing the same letter are not significantly different from each other, while those with different letters indicate statistically significant differences.

Coconut dairy alternative, with the lowest *TFC* (16.74 mg.l<sup>-1</sup>), reinforces the observation that coconut-based products typically have limited flavonoid content compared to other plant-based sources [12]. Higher *TPC* and *TFC* levels are linked to enhanced free radical scavenging activity, which significantly boosts the antioxidant properties of fermented milk [31]. The variability in *TPC* and *TFC* across plant-based dairy alternatives may also be attributed to differences in raw material processing, the starter cultures used for fermentation [32] and the production process [12]. For instance, the homogenisation process can break down cell walls and enhance the release of phenolics into the dairy alternative matrix; however, excessive heat can degrade these bioactive compounds [33].

Antioxidant activity can be evaluated using the ABTS<sup>•+</sup> radical-scavenging assay to determine total antioxidant capacity. Compared to the <sup>•</sup>DPPH radical-scavenging assay, all samples exhibited higher antioxidant activity in the ABTS<sup>•+</sup> assay (Fig. 1B). Notably, oat and soya-based dairy alternatives demonstrated superior antioxidant properties, likely due to their rich compositions of polyphenols and other bioactive compounds. These findings suggest that oat and soya-based dairy alternatives are more effective sources of antioxidants than coconut, almond, and cashew plant-based dairy alternatives.

## Colour

Colour is a key attribute of food because it is the first characteristic consumers notice. Colour influences consumers' acceptance of food and their expectations regarding quality, flavour, and freshness. It also serves as an indicator of safety, helping to identify spoilage or contamination. A visually appealing colour enhances consumer preference, making it essential to optimise for sensory appeal and market success [34]. Tab. 7 presents the lightness ( $L^*$ ), redness/greenness ( $a^*$ ), blueness/yellowness ( $b^*$ ), hue angle ( $H^\circ$ ), yellowness index (*YI*) and whiteness index (*WI*) values for different samples.

The  $L^*$  values are highest for the soya plant-based dairy alternative (80.98) and lowest for the cashew plant-based dairy alternative (77.58), which can be related to the particle size of the fat globules and protein [11]. The oat dairy alternative exhibits the strongest yellow hue (highest  $b^*$  and *YI* values) compared to other samples. The coconut dairy alternative is the whitest (highest *WI* and  $L^*$  values), making it visually lighter. The cashew dairy alternative shows the darkest tone (lowest  $L^*$  and *WI* values) with a moderate yellow hue. The soya dairy alternative has the brightest lightness ( $L^*$ ) and a balanced yellow-green hue. The almond dairy alternative has a subtle greenish tint (negative  $a^*$ ) and moderate lightness, yellowness, and whiteness attributes. Various factors can influence colour changes in dairy alterna-

**Tab. 7.** Colour parameters of the plant-based dairy alternatives.

	Coconut	Soya	Almond	Cashew	Oat
Lightness $L^*$	80.60 <sup>b</sup>	84.33 <sup>a</sup>	80.98 <sup>b</sup>	77.58 <sup>c</sup>	79.41 <sup>d</sup>
Redness/greenness $a^*$	-0.35 <sup>a</sup>	1.36 <sup>b</sup>	-0.64 <sup>c</sup>	1.48 <sup>d</sup>	2.02 <sup>e</sup>
Blueness/yellowness $b^*$	4.94 <sup>a</sup>	11.67 <sup>b</sup>	6.24 <sup>c</sup>	10.48 <sup>d</sup>	24.06 <sup>e</sup>
Hue angle $H^\circ$	94.54 <sup>b</sup>	96.63 <sup>a</sup>	95.82 <sup>a</sup>	82.14 <sup>c</sup>	85.40 <sup>d</sup>
Yellowness index	16.99 <sup>a</sup>	27.76 <sup>b</sup>	19.10 <sup>c</sup>	29.54 <sup>d</sup>	48.46 <sup>e</sup>
Whiteness index	27.22 <sup>a</sup>	1.68 <sup>b</sup>	21.77 <sup>c</sup>	-8.15 <sup>d</sup>	-42.60 <sup>e</sup>

Different small letters in superscript in the same row indicate statistically significant differences ( $p < 0.05$ ).

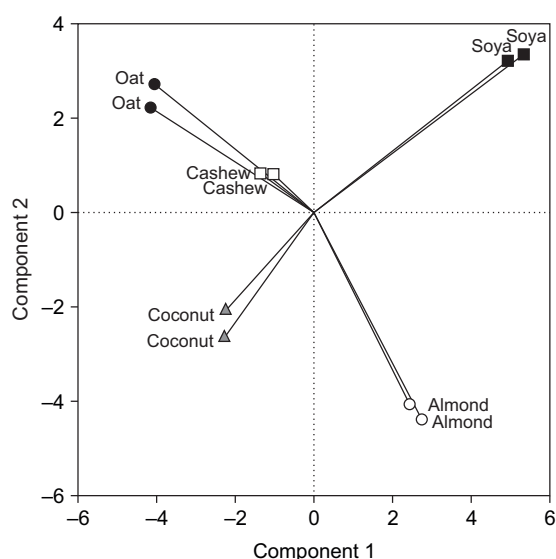
tives, including an extended fermentation process and the Maillard reaction [35]. These changes can impact the visual appeal and overall quality of the product, making it crucial to carefully control production conditions to maintain consistency.

### Statistical processing

Although ANOVA showed some statistical differences between individual plant-based dairy alternatives, from the information given above, practically none of the evaluated characteristics could alone serve as a marker for the purpose of plant-based dairy alternative discrimination according to the plant material. Therefore, the entire dataset of 29 experimental characteristics was statistically evaluated using multivariate statistics involving the methods of PCA, PCF and stepwise

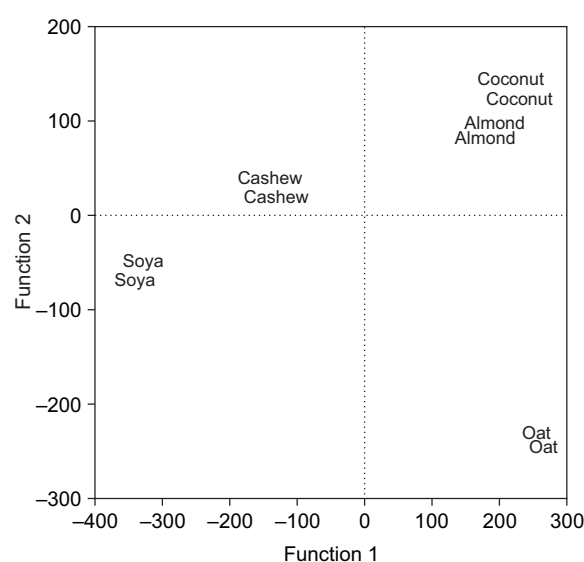
discriminant analysis. This model was suitable for visualising and classifying the samples under study according to their respective plant material.

PCA was performed to test the tendency of plant-based dairy alternatives to form groups and to describe the variability of the experimental characteristics. When PCA was applied to detect differences between dairy alternative samples, the original dataset was transformed into new variables, principal components. The results showed that the first two principal components (PCs) explained more than 69.5 % of the total variability. Fig. 2 shows that the dairy alternatives were discriminated into five separate clusters corresponding to the plant-based material. The descriptors of *TA*, *TFC*, octanoic acid and tetradecanoic acid played a dominant role in constructing



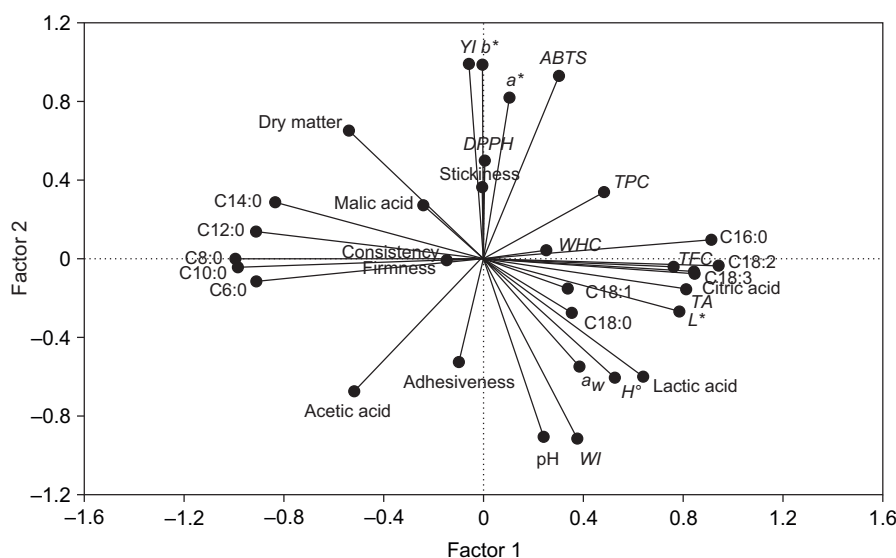
**Fig. 2.** Discrimination of plant-based dairy alternatives according to the plant material performed by principal component analysis.

All experimental characteristics were used as variables.



**Fig. 3.** Discrimination of plant-based dairy alternatives performed by stepwise discriminant analysis.

The entire dataset of experimental characteristics was used for discrimination.



**Fig. 4.** Plot of factors (varimax rotation)

indicating the importance of individual variables for plant-based dairy alternatives' discrimination.

$a_w$  – water activity,  $WHC$  – water holding capacity,  $TA$  – titratable acidity,  $TPC$  – total polyphenols concentration,  $TFC$  – total flavonoids concentration,  $ABTS$  – antioxidant activity determined by the ABTS assay,  $DPPH$  – antioxidant activity determined by the DPPH assay,  $L^*$  – lightness,  $a^*$  – redness/greenness,  $b^*$  – blueness/yellowness,  $H^\circ$  – hue angle,  $YI$  – yellowness index,  $WI$  – whiteness index.

the first PC, whereas antioxidant activity tested by  $ABTS^{*+}$ ,  $a^*$ ,  $YI$  and stickiness were the most important in constructing the second PC.

The discrimination of dairy alternatives by means of stepwise discriminant analysis using the whole dataset of experimental characteristics as discriminators achieved 100% correctness (Fig. 3). Regarding the influence of individual characteristics on the discrimination score, stepwise discriminant analysis proved the key role of  $TA$ ,  $b^*$ ,  $YI$ , octanoic and 9-octadecenoic acids in the discrimination of plant-based dairy alternatives under study. The importance of these parameters for discrimination was also confirmed by PCF with varimax rotation. Furthermore, the plot of factored data depicted in Fig. 4 indicates their importance for discrimination as well as mutual positive or negative correlations, e.g., positive correlations of antioxidant characteristics ( $TPC$  and  $ABTS$ ), textural properties (firmness and consistency), and/or  $TA$  with citric and lactic acid.

## CONCLUSIONS

This comprehensive comparative analysis of five commercially available plant-based dairy alternatives: soya, almond, cashew, oat, and coconut, demonstrated substantial differences in

their physicochemical, textural, nutritional, and antioxidant characteristics, which were strongly influenced by their plant-based raw materials and production formulations. The almond dairy alternative distinguished itself by the highest pH and exceptional  $WHC$ , indicating a smooth and creamy texture with minimal syneresis. In contrast, the oat dairy alternative, despite its higher acidity, maintained excellent  $WHC$  and notable firmness and consistency, making it a structurally robust product. The cashew dairy alternative exhibited the lowest  $WHC$  and weakest texture, suggesting limited consumer appeal in terms of mouth-feel and stability. The soya dairy alternative stood out for its high  $TA$  and lactic acid concentration, consistent with active fermentation, and exhibited strong antioxidant activity due to its rich phenolic and flavonoid content. The coconut dairy alternative, while lighter in colour and visually appealing, had comparatively lower antioxidant capacity. Fatty acid profiles highlighted the unique nutritional value of each product, such as MCFAs in coconut and polyunsaturated fatty acids in soya and almond.

Colorimetric analysis revealed distinguishable visual properties among samples, influenced by fermentation processes, formulation, and raw material composition. Multivariate statistical techniques, PCA, PCF, and stepwise discriminant

analysis, proved highly effective in discriminating the dairy alternative samples by plant source, with 100% classification accuracy. Key discriminators included *TA*, octanoic acid, 9-octadecenoic acid, flavonoid content, and antioxidant activity. Overall, the findings confirm that plant-based dairy alternatives are not only viable dairy alternatives but also offer distinct functional and nutritional benefits. The study provides a framework for the development of improved plant-based formulations tailored to specific consumer preferences and dietary needs, supporting both innovation and market diversification in the plant-based food sector.

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