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PHYSICO-CHEMICAL, ANTIOXIDANT AND SENSORY EVALUATION OF SPIRULINA-ENRICHED DRY-FERMENTED SAUSAGES WITH PROTECTIVE MICROBIAL CULTURES ADDITION

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ABSTRACT

Modern dietary preferences increasingly emphasize the incorporation of health-promoting components into conventional foods. Dry-fermented meat products, appreciated for their sensory attributes and prolonged shelf life supported by a higher microbial stability, present a suitable platform for functional enhancement. Spirulina (*Arthrospira platensis*), a cyanobacteria recognized for its rich nutritional profile, has shown potential as a valuable additive. This study evaluated the effect of adding spirulina powder at levels of 1% and 3% into dry-fermented sausages. This addition led to an increase in protein content of the sausages ($p \leq 0.05$). It was initially assumed that the pH would decrease due to the prebiotic effect of spirulina, which was expected to promote the growth of lactic acid bacteria and thus enhance lactic acid production. However, this hypothesis was not confirmed ($p \geq 0.05$), and no significant increase in lactic acid bacteria growth was observed either ($p \geq 0.05$). Colour measurements showed a greener colour in products with increasing amounts of spirulina ($p \leq 0.05$). Sensory analysis revealed that the acceptability of the product decreased with higher levels of spirulina addition ($p \geq 0.05$). These findings indicate that while spirulina can enhance the nutritional value of dry-fermented sausages, its addition should be balanced to ensure consumer's satisfaction. The addition of spirulina did not enhance the antioxidant capacity of the final product, which may be attributed to the low antioxidant potential of spirulina used in this study. As a result, no significant differences were found among the samples ($p \geq 0.05$).

Keywords: pH, texture, colour, antioxidant, chlorophyll, protective microbial culture

INTRODUCTION

Recent developments in the food industry have highlighted a growing interest in functional foods, generally defined as products, that in addition to their nutritional value, also have positive effects on human health by containing bioactive substances **(Frumuzachi *et al.*, 2025)**.
Dry-fermented meat products represent a traditional category of food characterized by specific sensory properties, long shelf life and a high level of microbial stability. Despite generally being characterized by a high content of salt, fat, saturated fatty acids and preservatives, their sustained popularity among consumers makes them a good target for product innovation (**Karwowski *et al.*, 2024**). The specific characteristics of dry-fermented sausages result from pH reduction, decreased water activity (with a maximum of 0.93 according to Decree No. 69/2016 Coll.), the addition of nitrates, nitrites and starter cultures and importantly smoking (**Braga *et al.*, 2025**). This controlled fermentation process is a complex of biochemical transformations involving acidification and the enzymatic degradation of proteins, lipids and carbohydrates resulting in the development of a characteristic aroma and flavour. These reactions rely on the activity of both endogenous enzymes naturally present in muscles and microbial enzymes derived from defined starter cultures (primarily lactic acid bacteria). Lactic acid bacteria play a crucial role in fermentation by using carbohydrates for production of lactic acid as a major metabolic product leading to a formation of a sour taste and significant reduction in pH, thereby enhancing a microbiological safety (**Zhao *et al.*, 2024; Fan *et al.*, 2025**). Efforts to increase the nutritional value of these products may lead to experimentation with the addition of various bioactive ingredients that could positively influence their overall composition (**Karwowski *et al.*, 2024**). One potential functional additive is spirulina (*Arthrospira platensis*), a nutrient-rich blue-green cyanobacteria (**Nabti *et al.*, 2023**). Its use in the meat industry could lead to an improvement in the nutritional profile and possibly influence the technological and sensory properties of the final product.

Spirulina (*Arthrospira platensis*) is a microscopic aqueous photoautotrophic cyanobacteria with an exceptional nutritional profile. Due to a rapid biomass production and ability to synthesize diverse bioactive compounds and macro- and micronutrients, spirulina is recognized as a highly promising and sustainable resource for the future (**Ramírez-Rodrigues *et al.*, 2021**). One of the key characteristics of this cyanobacteria is a high protein content, which accounts for approximately 60–70% of its dry weight. In addition, it is rich in essential amino and fatty acids, vitamins (especially B-vitamins, vitamin A, E and K), minerals (including iron, calcium, sodium, phosphorus, magnesium, and potassium), chlorophyll and antioxidants such as phycocyanin (**Shah *et al.*, 2024**). The addition of various plant-based ingredients may pose a risk to food safety. Although the use of unusual ingredients in recipes for reformulating meat products is attractive to manufacturers, their use should be accompanied by appropriate safeguards compatible with the HACCP system. An effective solution is the application of a combined microbial culture consisting of a starter culture (*Lactobacillus spp.*) and a protective microbial culture (*Lactobacillus curvatus*) (**Kameník *et al.*, 2024**).

Fermented spirulina represents a new progressive approach in creating functional foods, where the bioactive potential is increased due to the controlled action of microorganisms. During fermentation, the cellular structures of spirulina are broken down leading to a better availability or synthesis of different bioactive compounds responsible for increasing the overall antioxidant capacity (**Rutar *et al.*, 2021**). Its addition to dry-fermented meat products combines traditional products with modern nutritional trends, offering the opportunity to significantly improve the nutritional profile of these usually less healthy foods. The remaining question is the impact on quality parameters, especially sensory acceptability, being the main reason for conducting this study.

MATERIAL AND METHODS

Table 1 Recipe of sausages (kg.100 kg^{-1})

Ingredients	Control sausage
Beef lean meat	28.06
Pork lean meat	30.14
Pork fatty meat	18.10
Pork fat	20.00
Nitrite salt mixture	2.30
Spice mixture	1.40

Sausage production

Three sausage variants were prepared in two independent batches (10 kg each) according to the formulation presented in **Table 1**: a control (C) and two experimental groups containing 1% (S1) and 3% (S3) of spirulina powder (*Arthrospira platensis*) relative to the total weight of each group. Lean and fatty pork were sourced from a local slaughterhouse (Jatka Ivančice s.r.o., Czech Republic), delivered to the pilot plant at Mendel University's Department of Food Technology (CZ22067), and stored at <3 °C for 24 hours. Before processing, meat was portioned and cleaned of skin and tendons. Pork was classified as lean (≤5% fat) or fatty (≈50% fat), and coarsely minced (12 mm; TMP 23-98, Braher, Spain). The meat was mixed (RC-10, Manca, Spain) with the spice mixture Lovecký salám TN and nitrite salt (MASOPROFIT a.s., Czech Republic). At the final mixing stage, 0.25 g of starter culture Bitec SRSC01 (Gewürzmüller, Germany), together with protective microbial culture SafePro (*Lactobacillus curvatus*, Novonesis, Danemark) and spirulina powder (Zdraví s chutí s.r.o., Czech Republic) were incorporated to each batch, for S1 group 100 g of spirulina (1%), for S3 group 300 g of spirulina (3%), followed by fine mincing (8 mm). The meat batter was stuffed (HTS 95, HTS Fleischereimaschinen, Austria) into pork casings (30/32 mm; MASOPROFIT a.s., Czech Republic) and hand-hung on smoking racks. The racks were held at 20 °C for 24 hours. Subsequently, the samples were transferred to a smoking chamber (Bastramat B 850 FR, BASTRA GmbH, Germany) and subjected to an 8-hour smoking cycle using smoke at a temperature of 20 °C. Following the smoking process, the sausages were transferred to a ripening chamber (KRA-Gen3 M, Lackner, Germany) and subjected to a 7-day drying period at 20 °C and 88% relative humidity. The products were sampled for analysis and vacuum-packed (Boxer 35, Henkelman, Netherlands) one day after the completion of the 7-day drying process, and subsequently stored at 20 °C. Analyses were performed both one day after completion of the ripening process and at the end of the shelf life (21 days).

Chemical analysis of spirulina powder and sausages

Chemical composition analyses were conducted to determine the protein content (g.100 g^{-1}), fat content (g.100 g^{-1}), moisture content expressed as loss on drying (g.100 g^{-1}), and salt content (g.100 g^{-1}) of the samples. The samples were analysed at three time points: before the drying process, 1 day after the completion of the drying process and after 21 days of storage. For each experimental group, 250 g of homogenized material was used. The analyses were performed according to the official methods of the Association of Official Analytical Collaboration (AOAC) International: protein content (**AOAC, 2002**), fat content (**AOAC, 1996**), moisture content (**AOAC, 2005a**), and salt content (**AOAC, 2005b**). All analyses were performed on homogenized samples and carried out in triplicate to ensure accuracy and repeatability.

Weight loss during the drying process

The sausages were weighed at three defined technological stages:

- Day 0 – after filling into casings (initial weight)
- After smoking – at the end of the smoking stage
- After drying – at the end of the entire drying process

Each weighing thus represented a distinct processing phase rather than a single-day change. The total weight loss was calculated as the percentage of the initial product weight. All measurements were performed using a laboratory scale (Kern, Germany; accuracy ±0.01 g) and conducted in triplicate to ensure precision. These data served as the basis for interpreting the corresponding changes in moisture content and composition, reflecting the gradual dehydration of the sausages during smoking and drying.

Water activity

After the completion of the drying process and storage, the water activity of dry-fermented sausages was determined for cubes with a 0.5 cm edge using LabSwift-aw (NOVASINA AG, Switzerland). All these measurements were performed three times.

pH

The pH of all samples was measured using a calibrated pH meter HI981036 (Hanna Instruments, Rhode Island, USA) at three different time points: before the drying process, after the smoking process and after 21 days of storage. All measurements were performed in five replicates.

Texture properties

The texture properties of the sausages were analysed 1 day after the drying process and after 21 days of storage using a TIRATEST 27 025 texturometer (TIRA Maschinenbau GmbH, Germany). Prior to measuring, all samples were brought to a room temperature (20 °C). The measurement was carried out with the use of a Meullenet-Owens Razor Shear (MORS), applying a constant crosshead speed of 50 mm/min, and penetrating the sample to a depth of 10 mm. Each group of samples was measured at least 10 times.

Colour measurement

The colour of the samples was measured 1 day after the drying process and 21 days of storage using a CM-3500d spectrophotometer (Konica Minolta, Japan) in the CIELAB colour space, which includes parameters $L*$ (lightness), $a*$ (redness) and $b*$ (yellowness). Measurements were made both on the surface and in the cut (D 65, 6 500 K) with SCE (Specular Component Excluded) mode and an 8 mm diameter aperture. Surface values were taken before the drying process, then 1 day after drying, and again after 21 days of storage, both on surface and in the cut, to accurately observe the differences in colour characteristics between the surface and the interior of the product. Colour was measured both on the surface and in the cut of the sausages to differentiate the visual characteristics of the casing and interior. At each stage, a minimum of three independent measurements were taken from different areas of each sample, and the mean values were used for statistical evaluation.

Antioxidant capacity

The antioxidant capacity was evaluated using the spectrophotometric phosphomolybdenum method for both the spirulina powder and the dry-fermented sausage samples at the end of the storage period. The analytical procedure was optimised for the purposes of this study, based on the method described by **Alam *et al.* (2013)**. Trolox was used as a reference standard, and a calibration curve was constructed based on a series of known Trolox concentrations, with the corresponding absorbance values measured spectrophotometrically. The antioxidant capacity of the samples was expressed as Trolox equivalents, calculated from the absorbance values of the sample extracts using the regression equation obtained from the Trolox calibration curve. For the assay, 100 µl of sample extract (prepared using 80% methanol) or 100 µl of Trolox calibration solution was combined with 1 ml of reagent solution. The reagent was prepared by dissolving 0.046 g of sodium phosphate and 0.049 g of ammonium molybdate in 10 ml of distilled water, followed by the addition of 300 µl of concentrated sulfuric acid. The blank solution consisted of 100 µl of 80% methanol and 1 ml reagent. All mixtures were incubated at 95 °C for 60 minutes and then cooled down to room temperature. The absorbance was measured at 695 nm using a spectrophotometer (CM-3500d, Konica Minolta, Japan). Each sample was measured as triplicate.

Microbiological analysis

Microbiological analysis of the sausage samples was determined 1 day after the completion of the drying process and after 21 days of storage at a controlled temperature of 20 °C. The analytical procedures were performed in accordance with the standardized methodology described by **Kalhotka *et al.* (2012)**. Total aerobic counts were determined on PCA agar (Biokar Diagnostics, France) incubated at 30 °C for 72 hours.

Sensory analysis

Sensory evaluation was conducted at the accredited sensory laboratory of Mendel University in Brno, Department of Food Technology, designed in accordance with ISO 8589 standards. The evaluations took place in isolated, uniformly lit booths to eliminate external influences and ensure objective assessment. A trained panel consisting of ten assessors, qualified under ISO 8586-1, carried out the sensory assessment. The evaluation was performed after the drying process and again after 21 days of vacuum storage, in order to assess both the immediate sensory quality and the stability of sensory attributes during storage. Prior to the assessment, the assessors were briefed on the sensory assessment procedure. A total of seven hedonic descriptors were evaluated: colour, texture, aroma, taste, acidity, overall appearance, and overall acceptability. Unstructured graphical scales of 100 mm were used, with the extremes marked 0 (unsatisfactory) and 100 (excellent). Bakery products and tap water were provided as palate cleansers. Samples were prepared by removing the outer ends (3 cm from each side) of the sausages, retaining only the central portion for analysis. Slices of 2 mm thickness were obtained using a slicing machine, equilibrated to room temperature (20 °C) prior to serving, and evaluated without delay.

Statistical analysis

Data normality was assessed using the Shapiro–Wilk test. Group differences were analysed by one-way ANOVA followed by Tukey's post-hoc test. Statistical significance was set at $p \leq 0.05$. Analyses were performed in STATISTICA version 14 (TIBCO, USA).

RESULTS AND DISCUSSION

Chemical analysis of spirulina powder and sausages

Table 2 Chemical analysis of spirulina powder (g.100 g^{-1})

	SP (x̄±*SD*)
Water	3.60±0.14
Fat	5.70±0.09
Protein	64.30±0.33
NaCl	0.90±0.11

Legend: SP = spirulina powder, x̄±*SD* = average ± standard deviation

The proximate composition of the commercially obtained spirulina powder is shown in **Table 2**. Based on the chemical analysis, the spirulina powder contained a high proportion of protein, confirming its potential as a valuable protein source (**Shah *et al*., 2024**).

Table 3 Chemical analysis of sausages with spirulina powder before the drying process (g.100 g^{-1})

	C (x̄±*SD*)	**S1** (x̄±*SD*)	**S3** (x̄±*SD*)
Water	51.84±0.28^{a}	50.47±0.27^{b}	49.90±0.09^{c}
Fat	24.68±0.36	24.83±0.23	24.39±0.16
Protein	18.58±0.48^{c}	19.43±0.46^{b}	21.91±0.57^{a}
NaCl	2.98±0.03	3.06±0.04	3.09±0.01

Legend: C = control without spirulina powder, S1 = with 1% addition of spirulina powder, S3 = with 3% addition of spirulina powder, x̄±*SD* = average ± standard deviation, $^{a–c}$ different letters within the same row indicate statistically significant differences ($p≤0.05$)

The addition of spirulina affected the composition of the sausages prior to the drying process (**Table 3**). In samples collected before drying, a statistically significant increase in protein content was observed ($p≤0.05$). Since spirulina was added in its dry powdered form, an increase in dry matter content was expected. According to **Tańska *et al.* (2017)**, spirulina has the ability to absorb water, which may explain why sausages with added spirulina showed lower water content per 100 g compared to the control ($p≤0.05$), although the same amount of water was added to all samples.

Table 4 Chemical analysis of dry-fermented sausages with spirulina powder with different storage time (g.100 g^{-1})

	Days of storage	**C** (x̄±*SD*)	**S1** (x̄±*SD*)	**S3** (x̄±*SD*)
Water	1	36.21±0.19^{a}	35.19±0.48^{b}	33.44±0.27^{c}
	21	35.02±0.14^{a}	33.32±0.39^{b}	30.54±0.23^{c}
Fat	1	34.89±0.08	33.75±0.26	33.50±0.35
	21	33.69±0.11	32.98±0.21	33.01±0.29
Protein	1	23.22±0.28^{c}	24.25±0.56^{b}	25.95±0.74^{a}
	21	23.35±0.19^{c}	25.09±0.48^{b}	26.97±0.52^{a}
NaCl	1	3.40±0.02	3.36±0.03	3.39±0.02
	21	3.37±0.05	3.31±0.04	3.38±0.04

Legend: C – control without spirulina powder, S1 – with 1% addition of spirulina powder, S3 – with 3% addition of spirulina powder, x̄±*SD* – average ± standard deviation, $^{a–c}$ different letters within the same row indicate statistically significant differences ($p≤0.05$)

The aforementioned trend, characterized by an increase in protein content as a result of spirulina addition and a simultaneous reduction in moisture content in the products (**Table 4**), remained statistically significant even after the completion of the drying process ($p≤0.05$). As anticipated, increasing levels of spirulina addition resulted in a decrease in moisture content, with the lowest value observed in the variant containing the highest proportion of spirulina ($p≤0.05$). Concurrently, the effect of spirulina on the protein content of the product was also confirmed ($p≤0.05$). Due to the high protein content in spirulina (**Shah *et al*., 2024**), the S3 variant exhibited the highest measured protein content compared to the other variants ($p≤0.05$). After 21 days of vacuum storage at 20 °C (**Table 4**), no significant changes in the chemical composition of dry-fermented sausages were observed compared to 1 day after the drying process ($p≥0.05$).

Weight loss during the drying process

Weight loss was evaluated in sausages after smoking, after the drying process, and after 21 days of storage. The lowest weight loss was observed in sample S3 after smoking process (7.33%±0.21) ($p≤0.05$) compared to sample C (13.50%±0.18) and S1 (14.14%±0.34), indicating a potential influence of spirulina on moisture retention (**Boukhari *et al.*, 2018**). After 7 days of drying, weight loss reached 19.43%±0.64 in sample C, 19.75%±0.39 in sample S1, and 19.77%±0.43 in sample S3. During the 21-day storage period, only minimal additional weight loss was observed, with an average decrease of approximately 0.10% ($p≥0.05$). Weight values recorded after the completion of the drying process and after 21 days of storage did not differ significantly, indicating that vacuum packaging effectively prevented further moisture loss. These results are consistent with the expected gradual dehydration occurring during the smoking and drying phases of fermented sausages and confirm the data presented in Table 3, reflecting a standard technological process rather than an experimental deviation.

Water activity

Table 5 Water activity of dry-fermented sausages with spirulina powder with different storage time

	C (x̄±*SD*)	**S1** (x̄±*SD*)	**S3** (x̄±*SD*)
1 day of storage	0.92±0.02	0.93±0.02	0.93±0.03
21 days of storage	0.92±0.01	0.92±0.01	0.92±0.02

Legend: C – control without spirulina powder, S1 – with 1% addition of spirulina powder, S3 – with 3% addition of spirulina powder, x̄±*SD* – average ± standard deviation

The drying process not only reduced the overall moisture content but also significantly decreased the water activity (Table 5), thereby making the dry-fermented sausages less susceptible to the growth of undesirable microorganisms (Mudadu et al., 2022). This reduction in water activity contributes to improved microbial stability and consequently extends the shelf life, allowing the sausages to be stored for longer periods without spoilage (Patarata et al., 2022). The final water activity values after 21 days of storage were consistent between samples C (0.92 ± 0.01) and S1 (0.92 ± 0.01), while S3 exhibited a slightly lower value (0.92 ± 0.02). These results meet the legal threshold for shelf-stable dried meat products. In agreement with the findings reported by Kročko et al. (2024) for other natural additives, the incorporation of spirulina did not affect the drying or aging behavior of the fermented sausages. According to Czech Decree No. 69/2016 Coll. (Ministry of Agriculture of the Czech Republic, 2016), fermented sausages must have a water activity of 0.93 or lower to be classified as shelf-stable and dried.

pH

Table 6 pH of sausages with spirulina powder

	C (x̄±*SD*)	**S1** (x̄±*SD*)	**S3** (x̄±*SD*)
Before the drying process	5.72±0.04	5.72±0.03	5.72±0.04
After the drying process	5.50±0.06	5.62±0.05	5.63±0.05
1 day of storage	4.76±0.02	4.87±0.03	4.88±0.01
21 days of storage	4.81±0.02	4.90±0.03	4.91±0.01

Legend: C – control without spirulina powder, S1 – with 1% addition of spirulina powder, S3 – with 3% addition of spirulina powder, x̄±*SD* – average ± standard deviation

A decrease in pH during the ripening process (**Table 6**) was expected due to an ongoing fermentation, and this trend was confirmed across all sausage samples. It was also anticipated that sausages with added spirulina would show a greater decrease in pH compared to the control sample. This assumption was based on the idea that spirulina could act as a prebiotic, promoting the growth of lactic acid bacteria (**Elwakil *et al*., 2024**). However, this expected enhanced acidification in the spirulina-enriched samples was not observed. On the contrary, the control samples without spirulina addition showed a slightly lower pH. The pH reduction in spirulina-enriched samples did not significantly differ from that of the control ($p≥0.05$), suggesting that spirulina did not exert a noticeable prebiotic effect under the given conditions.

Texture properties

Table 7 Textural analysis of dry-fermented sausages with spirulina powder with different storage time (N)

	C (x̄±*SD*)	**S1** (x̄±*SD*)	**S3** (x̄±*SD*)
MORS: 1 day of storage	8.22±1.21	7.51±1.22	7.09±1.04
MORS: 21 days of storage	6.68±1.79	6.25±0.89	6.64±1.65

Legend: C – control without spirulina powder, S1 – with 1% addition of spirulina powder, S3 – with 3% addition of spirulina powder, x̄±*SD* – average ± standard deviation

Textural analysis using the MORS method (**Table 7**) did not reveal any statistically significant effect of spirulina addition on the surface firmness of the analysed meat products ($p≥0.05$). However, after 21 days of storage, a decrease in shear force values was observed across all samples. This reduction is likely attributable to partial rehydration of the sausage surface during storage in vacuum packaging. The vacuum environment may have facilitated moisture migration from the inner layers toward the surface, resulting in a softened texture over time (**Stasiewicz *et al*., 2014**).

Colour measurement

Table 8 Colour analysis of sausages with spirulina powder before the drying process

	C (x̄±*SD*)	**S1** (x̄±*SD*)	**S3** (x̄±*SD*)
L^* (surface)	33.30±1.78^{a}	27.80±2.35^{b}	26.92±0.96^{b}
a^* (surface)	13.55±1.06^{a}	-1.22±0.21^{b}	-3.10±0.12^{c}
b^* (surface)	12.04±3.43^{a}	3.77±0.46^{b}	3.87±0.39^{b}

*L** (cut)	33.78±2.40[a]	33.40±2.42[a]	25.67±0.60[b]
*a** (cut)	14.95±1.43[a]	-5.05±1.17[b]	-4.51±1.21[b]
*b** (cut)	10.20±1.32[a]	4.79±0.71[b]	3.78±0.30[b]

Legend: C – control without spirulina powder, S1 – with 1% addition of spirulina powder, S3 – with 3% addition of spirulina powder, x̄±*SD* – average ± standard deviation, [a–c] different letters within the same row indicate statistically significant differences ($p \leq 0.05$)

Table 9 Colour analysis of dry-fermented sausages with spirulina powder with different storage time

	Days of storage	C (x̄±*SD*)	S1 (x̄±*SD*)	S3 (x̄±*SD*)
*L** (surface)	1	29.28±1.32[a]	23.16±0.39[b]	24.71±0.93[b]
	21	28.06±2.01[a]	22.93±0.04[b]	23.67±0.01[b]
*a** (surface)	1	7.11±1.44[a]	-0.66±0.16[b]	-1.07±0.10[c]
	21	8.20±1.50[a]	-0.59±0.02[b]	-0.46±0.03[b]
*b** (surface)	1	8.55±1.69[a]	2.41±0.12[b]	2.62±0.25[b]
	21	9.80±2.19[a]	2.81±0.03[b]	2.23±0.06[b]
*L** (cut)	1	43.32±7.84[a]	30.68±1.67[b]	29.89±1.24[b]
	21	41.97±0.02[a]	26.40±0.06[b]	25.21±0.06[b]
*a** (cut)	1	13.01±2.70[a]	-2.24±1.07[b]	-2.15±1.09[b]
	21	12.17±0.03[a]	-0.48±0.01[b]	-0.60±0.04[b]
*b** (cut)	1	6.96±2.23[a]	1.89±0.58[b]	1.92±0.19[b]
	21	5.63±0.03[a]	1.28±0.01[b]	1.14±0.02[b]

Legend: C – control without spirulina powder, S1 – with 1% addition of spirulina powder, S3 – with 3% addition of spirulina powder, x̄±*SD* – average ± standard deviation, [a–c] different letters within the same row indicate statistically significant differences ($p \leq 0.05$)

The colour evaluation of sausages enriched with spirulina revealed expected differences compared to the control sample, primarily due to the natural green hue of spirulina (**Shah *et al*., 2024**). As anticipated, sausages containing spirulina (S1 and S3) exhibited a greener appearance than the control, attributed to the presence of blue and green pigments in spirulina, notably phycocyanin and chlorophylls **(Luo *et al*., 2018**). However, no significant differences in colour parameters were observed between the two spirulina-enriched variants. Following the drying process and throughout 21 days of storage, all products became darker in colour compared to their initial state post-production. The observed darkening may be caused by formation of dark-coloured compounds resulting from the oxidation of phenolic substances present in spirulina powder (**Luo *et al*., 2018**). Before the drying process (**Table 8**), S1 and S3 showed statistical differences in the *a** (surface) value ($p \leq 0.05$), with S3 exhibiting a greener colour, and in the *L** (cut) value ($p \leq 0.05$), where S1 appeared lighter. One day after the drying process (**Table 9**), the only significant difference between the spirulina samples was again in the *a** (surface) parameter ($p \leq 0.05$), with S3 maintaining a darker greener shade. After 21 days of storage (**Table 9**), no statistically significant colour differences were detected between the spirulina-enriched samples ($p \geq 0.05$), indicating consistent colour stability over time.

Antioxidant capacity

Table 10 The values of total antioxidant capacity expressed as Trolox Equivalent (µmol.g[-1])

	SP (x̄±*SD*)	C (x̄±*SD*)	S1 (x̄±*SD*)	S3 (x̄±*SD*)
Trolox Equivalent	89.16±6.80	33.76±0.38[b]	39.18±1.76[a]	33.96±2.69[b]

Legend: SP – spirulina powder, C – control without spirulina powder, S1 – with 1% addition of spirulina powder, S3 – with 3% addition of spirulina powder, x̄±*SD* – average ± standard deviation, [a–c] different letters within the same row indicate statistically significant differences ($p \leq 0.05$)

The antioxidant capacity analysis (**Table 10**) revealed that the sample with 1% addition of spirulina powder (S1) exhibited a significantly higher antioxidant capacity compared to the control (C) ($p \leq 0.05$), suggesting a potential benefit of moderate spirulina supplementation. Interestingly, the 3% spirulina sample (S3) did not differ significantly from the control ($p \geq 0.05$) and showed significantly lower antioxidant capacity than S1 ($p \leq 0.05$). This unexpected trend might be attributed to several factors, including sample inhomogeneity, possible degradation of bioactive compounds at higher concentrations, or interactions between spirulina components and the food matrix, which could have reduced the accessibility or activity of antioxidant compounds (**Stunda-Zujeva *et al*., 2023**).

Moreover, the spirulina powder used in this study exhibited a notably lower antioxidant capacity compared to values reported in published literature. For example, **Durďáková *et al.* (2024)** reported antioxidant capacities of spirulina exceeding 150 mmol Trolox equivalent per gram using the same analytical method, which is substantially higher than the value observed in our study. These findings suggest that the spirulina may have been of lower quality, or that its antioxidant potential may have been reduced, possibly due to inappropriate processing, handling, or storage conditions. This could have contributed to the degradation of sensitive bioactive compounds, thereby reducing the antioxidant potential (**Papalia *et al.*, 2019**).

Microbiological analysis

Table 11 Microbiological evaluation of sausages with spirulina powder (log_{10}CFU.g[-1])

	Days of storage	C (x̄)	S1 (x̄)	S3 (x̄)
Total plate count	1	**8.21**	**8.14**	**8.19**
	21	8.42	8.36	8.65

Legend: C – control without spirulina powder, S1 – with 1% addition of spirulina powder, S3 – with 3% addition of spirulina powder, x̄ – average

Although spirulina was considered a potential nutrient-rich source capable of promoting the growth of lactic acid bacteria, microbiological analysis (Table 11) showed that this expected effect was not reflected in the total plate count, which did not show a statistically significant increase compared to the control samples ($p \geq 0.05$), indicating that spirulina did not significantly affect any other microbiota of the fermented product ($p \geq 0.05$). Furthermore, no significant changes were observed in the microbial counts during storage, suggesting that the microbial stability of the products was maintained throughout the storage period ($p \geq 0.05$).

Sensory analysis

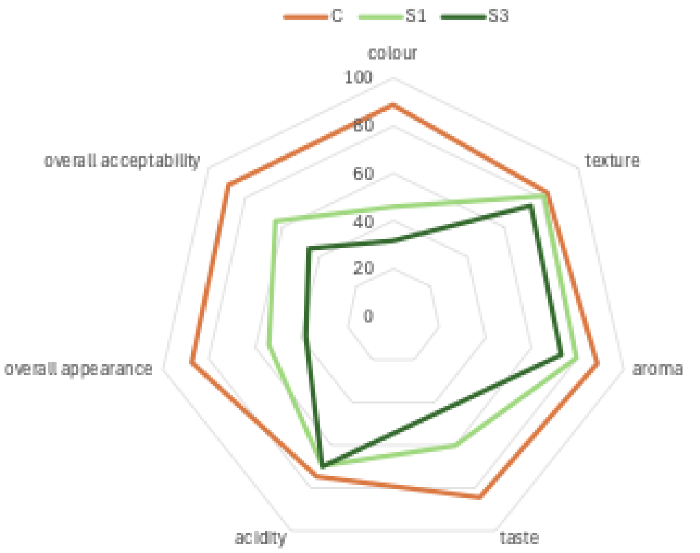

Figure 1 Hedonic sensory evaluation of sausages with spirulina powder 1 day after the drying process

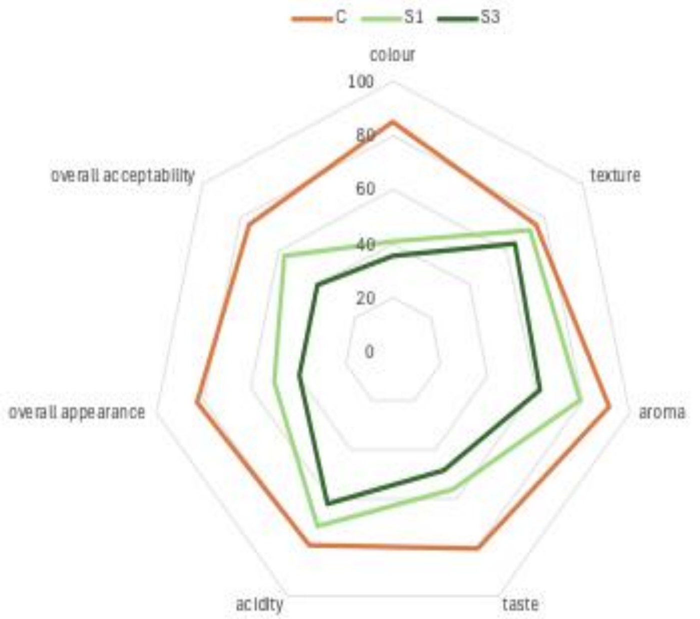

Figure 2 Hedonic sensory evaluation of sausages with spirulina powder after 21 days of storage

In the sensory analysis, the sausages with added spirulina received the lowest scores among all samples. A clear negative correlation was observed between the amount of spirulina added and the overall sensory acceptability – the higher the spirulina content, the lower the sensory evaluation. One day after the drying process (**Figure 1**), the control sample without spirulina was rated the best in overall acceptability. In contrast, spirulina-enriched samples received significantly lower scores ($p \leq 0.05$), particularly for attributes such as colour, aroma and taste. Similarly, to the findings of this study, **Ghaly *et al*. (2015)** also reported that higher amounts of spirulina reduced the acceptability of the final product, most notably in terms of taste perception. The negative ratings in this research were likely due to the high levels of spirulina used, which may have adversely influenced the sensory profile of the products. After 21 days of storage (**Figure 2**), the sensory values remained relatively stable, with no significant changes in the ratings ($p \geq 0.05$). As higher levels of spirulina led to a decreased overall acceptability of the sausages, using a lower amount could be more suitable for ensuring a better sensory profile. The addition of a plant-based ingredient does not necessarily need to have a positive effect on quality parameters. The basic premise and condition are that it does not affect the hygienic quality characteristics. The addition of a plant-based ingredient with a nutritional effect is a reformulation strategy aimed to maintain food safety with minimal impact on quality parameters. If a change in colour is expected with the addition of a plant-based ingredient, this may not be perceived negatively by consumers if the descriptors essential for the acceptability of the meat product are not critically affected (**Mesárošová *et al*., 2024**). These descriptors are most often texture, aroma, and taste or the overall acceptability of the food (**Stajić *et al*., 2024**). However, further research is needed to confirm or demonstrate the full usability of adding certain non-native components to the recipes (**Mesárošová *et al*., 2024**). Using a protective microbial culture alongside a starter culture is sensible when quality parameters are not affected and the potential hygiene risk is reduced (**Kameník *et al*., 2024**).

CONCLUSION

The addition of spirulina into dry-fermented sausages influenced certain quality parameters. As expected, the protein content increased due to the naturally high protein content in spirulina. On the contrary to initial assumptions, no significant changes were observed in pH values and total plate count. Shear force remained similar across samples, with a decrease after 21 days likely due to water migration towards the surface during vacuum packaging. Spirulina caused significant changes in colour parameters L^*, a^*, b^* at all stages (before drying, 1 day after the drying process and after 21 of storage) attributed to its green pigmentation. Despite its reported antioxidant potential, no significant antioxidant capacity was found in the products regardless of spirulina amount. Sensory evaluation showed decreased acceptability with increasing spirulina content. Optimized properties of the final product might be achieved by incorporating higher-quality spirulina powder, possibly resulting in higher antioxidant capacity and improved functional and microbiological properties of the product. It is therefore crucial to emphasize that the quality of spirulina play a significant role in determining its effectiveness. The quality is largely influenced by cultivation as well as post-harvest process such as drying method and storage, which can greatly affect its nutritional value and bioactive compound content. Although the addition of spirulina powder did not significantly influence the technological parameters of sausage production, it negatively influenced sensory attributes, resulting in reduced consumer acceptability.

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