processes

MDPI

Article

Portulaca oleracea as a Functional Ingredient in Organic Cooked Frankfurters: A Sustainable Approach to Shelf-Life Extension and Oxidative Stability Without Synthetic Nitrites

Kadyrzhan Makangali, Gulnazym Ospankulova, Gulzhan Tokysheva *, Aknur Muldasheva and Kalamkas Dairova

Department of Technology of Food and Processing Industries, S. Seifullin Kazakh Agrotechnical Research University, Astana 010000, Kazakhstan; k.makangali@kazatu.kz (K.M.); gulnazymospankulova2000@gmail.com (G.O.); a.muldasheva@kazatu.kz (A.M.); kalamkasdairova685@gmail.com (K.D.)

* Correspondence: tokishevagulzhan@gmail.com; Tel.: +7-701-667-05-91

Abstract

Consumer demand for organic and nitrite-free meat products has stimulated the search for sustainable alternatives to synthetic curing agents. Conventional nitrites are effective in stabilizing color, inhibiting lipid oxidation, and suppressing pathogens, but their use raises health concerns due to potential nitrosamine formation. This study investigated the application of *Portulaca oleracea* powder as a multifunctional ingredient to fully replace sodium nitrite in organic cooked frankfurters. Two formulations were produced: control frankfurters with sodium nitrite and experimental frankfurters with purslane powder 1.2%. Physicochemical, oxidative, proteomic, and antioxidant parameters were monitored during refrigerated storage. Purslane incorporation improved the lipid profile by increasing α-linolenic acid and lowering the ω-6/ω-3 ratio, while peroxide, thiobarbituric acid reactive substances (TBARS), and acid values remained significantly lower than in nitrite-containing controls after 10 days. Protein oxidation was also reduced, and SDS-PAGE profiles confirmed that the major structural muscle proteins remained stable, indicating that purslane addition did not disrupt the core proteome. Antioxidant assays showed strong ferric-reducing antioxidant power (FRAP) activity 13.7 mg GAE/g and enhanced 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging capacity 22.3%, highlighting purslane's contribution to oxidative stability. Although redness (a*) was lower than in nitrite controls, overall color stability (L*, b*) remained high. Taken together, purslane enhanced oxidative stability and quality attributes of nitrite-free organic frankfurters; microbiological validation is ongoing and will be reported separately.

Keywords: organic frankfurters; *Portulaca oleracea*; nitrite-free meat products; oxidative stability; antioxidant capacity; functional ingredients; sustainable food engineering

Academic Editors: Jung-Feng Hsieh and Jooyeoun Jung

Received: 10 September 2025
Revised: 30 September 2025
Accepted: 2 October 2025
Published: 5 October 2025

Citation: Makangali, K.; Ospankulova, G.; Tokysheva, G.; Muldasheva, A.; Dairova, K. *Portulaca oleracea* as a Functional Ingredient in Organic Cooked Frankfurters: A Sustainable Approach to Shelf-Life Extension and Oxidative Stability Without Synthetic Nitrites. *Processes* **2025**, *13*, 3167. https://doi.org/10.3390/pr13103167

1. Introduction

Consumer demand for organic and clean-label foods free from synthetic additives has significantly increased in recent years, driven by health awareness and sustainability concerns [1]. In organic meat processing, this shift poses major challenges due to restrictions on the use of chemical preservatives, particularly nitrites [2]. Nitrites are central

to conventional curing as they stabilize the red–pink color, develop flavor, inhibit lipid oxidation [3,4]. However, nitrites may react with amines to form N-nitrosamines (NAs), which have been associated with carcinogenic effects [5]. The European Food Safety Authority has highlighted concerns about dietary nitrite exposure and the risk of nitrosamine formation [6].

A broad range of natural alternatives has been studied, including vitamins, plant extracts, herbs, and fruits, due to their antioxidant and bacteriostatic properties [7,8]. Extracts rich in betalains, flavonoids, and phenolic acids have shown potential for both color stabilization and oxidative protection [9,10]. Other approaches include nitrate-rich vegetable extracts combined with starter cultures for in situ nitrite formation [11] or advanced processing such as high-pressure processing (HPP) for shelf-life extension [12]. Still, no solution fully replicates the multifunctionality of nitrites, especially in organic sausages [13].

Portulaca oleracea (purslane) has emerged as a promising plant-based alternative. Purslane is abundant in omega-3 fatty acids, flavonoids, alkaloids, betalains, and polysaccharides, contributing to strong antioxidant, antimicrobial, and anti-inflammatory activities [14,15]. It has been successfully applied in dairy, bakery, and packaging systems, where it enhanced oxidative stability and nutritional properties [16]. In meat systems, purslane supplementation has been shown to increase ferric-reducing antioxidant power (FRAP) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, while reducing peroxide and thiobarbituric acid values [17,18]. These results demonstrate its capacity to extend shelf life and maintain product quality under refrigerated storage.

Nevertheless, uncertainties remain. While some studies attribute the protective effects of purslane primarily to phenolic radical scavenging, others emphasize metal chelation, betalain-driven antioxidation, or interactions with fatty acids [19,20]. The lack of consensus underscores the need for systematic evaluation in meat systems, particularly in nitrite-free organic sausages, where preservation is technologically challenging [21]. In this study, we prioritized a system-level evaluation under nitrite-free conditions (lipid/protein oxidation endpoints, color stability, fatty-acid profile, and safety indices) to determine whether purslane can functionally replace nitrite in organic frankfurters. While we report multiple oxidative markers (peroxide value, TBARS, FRAP, DPPH) and proteomic fingerprints, mechanistic deconvolution of individual antioxidant pathways (phenolic radical scavenging vs. metal chelation vs. betalain-driven effects or fatty-acid interactions) was beyond the scope of the present work and is addressed in the Discussion Section as a limitation and outlook. The objective of this study was therefore to develop organic cooked sausages in which *Portulaca oleracea* powder fully replaces sodium nitrite. In this study, we assessed the effects of purslane addition on physicochemical parameters, oxidative stability, fatty-acid profile, color development, and sensory attributes during refrigerated storage.

Building on prior studies that used purslane in meat systems and broader strategies for nitrite replacement, this work demonstrates, for the first time to our knowledge, a full nitrite replacement in organic cooked frankfurters using a whole-plant purslane powder 1.2% as a single, clean-label ingredient. We couple comprehensive functional endpoints-primary/secondary lipid oxidation, hydrolytic rancidity, protein carbonyls, pre/post-light color stability, and fatty-acid profiling-with SDS-PAGE evidence of plant markers without perturbing the core muscle proteome. This system-level validation in an organic, nitrite-free matrix addresses the technological challenge of shelf-life extension and safety without synthetic nitrites, while explicitly delineating mechanistic questions for future targeted studies.

2. Materials and Methods

2.1. Materials

Organic cooked frankfurters were prepared using fresh beef as the primary raw material. The visual appearance of the final products is presented in Figure 1. Carcasses were deboned, trimmed, and connective tissues removed, after which the meat was ground through a plate with a particle size of 2–8 mm. The minced beef was salted and subjected to maturation at 4 °C for 18 h. Two different formulations were produced: Control formulation: beef frankfurters prepared with sodium nitrite (as conventionally applied in curing); Experimental formulation: beef frankfurters supplemented with *Portulaca oleracea* L. powder (1.2% of the total batter weight) as a natural alternative to nitrite. The purslane inclusion level of 1.2% was selected based on pilot trials, where higher concentrations (>1.5%) imparted bitterness and negatively affected sensory quality (unpublished data). The detailed formulations of both control and experimental frankfurters are summarized in Table 1.

In both cases, the batter was chopped (12 min), stuffed into natural casings, rested at 2–8 °C for 2 h, roasted at 85–95 °C until the internal temperature reached 45 °C, cooked at 75–85 °C until the internal temperature reached 72 °C, and cooled to an internal temperature of 15 °C prior to refrigerated storage. All thermal processing steps were carried out in a universal thermal chamber KTD-100 (Initsiativa, Moscow, Russia) in accordance with hygienic requirements for organic meat production, following Codex Alimentarius CAC/RCP 58-2005 (Code of Hygienic Practice for Meat), ISO 22000:2018 (Food safety management systems) [22], and the national standard ST RK 3111-2023 "Organic products. Requirements for production, storage, transportation, and sale [23].

Figure 1. Visual appearance of organic cooked frankfurters: (**left**) control sample with sodium nitrite; (**right**) experimental sample with *Portulaca oleracea* (purslane) powder.

Table 1. Formulations of organic cooked frankfurters (%, w/w).

Ingredient	Control (With Sodium Nitrite)	Experimental (With Purslane Powder)
Beef (lean, deboned)	76.0	76.0
Water/ice	8.0	8.0
Salt (NaCl)	1.5	1.5
Sodium nitrite	0.015	–
Portulaca oleracea powder	–	1.2
Spices (pepper, nutmeg, etc.)	1.0	1.0
Starch (potato/tapioca)	2.0	2.0
Onion and carrot (minced)	10.0	10.0

Fresh aerial parts of *Portulaca oleracea* L. (purslane) were collected in the Kyzylorda region, Kazakhstan. The plant material was washed, air-dried, and subjected to convective drying at 45 ± 2 °C until constant weight. The dried material was ground in a laboratory mill and sieved to obtain a powder fraction <250 μm. The powder was vacuum-packed and stored at 4 °C until use.

2.2. Fatty Acid Composition Analysis

Fatty acid methyl esters (FAMEs) were determined using a gas chromatograph (Agilent 7890, Agilent Technologies, Andover, MA, USA) equipped with a flame ionization detector (FID) and an HP-Innowax capillary column (60 m × 0.32 mm × 0.5 μm). Nitrogen was used as the carrier gas. The oven program was set from 100 °C to 260 °C with an increment of 10 °C/min. One microliter of each sample was injected with a split ratio of 1:100. Detector temperatures were maintained between 250 and 300 °C. Identification of fatty acids was performed by comparing retention times with a commercial FAME standard mixture (Supelco No. 47885U, C6–C24), and quantification was carried out using the internal standard method.

2.3. Color Measurement

The surface color of frankfurters samples was evaluated using a spectrophotometer (Konica Minolta CM-2300d, Osaka, Japan). The procedure followed the methodology described by Iftikhar et al. [24], previously applied to assess antioxidant activity in minced beef systems. Before measurement, the instrument was calibrated against both a white standard and zero calibration tiles. Each reported value represents the arithmetic mean of five replicate measurements per sample.

2.4. Protein Fraction Characterization by One-Dimensional Electrophoresis

Protein profiles were analyzed using one-dimensional polyacrylamide gel electrophoresis (SDS-PAGE). Approximately 100 mg of each sample was homogenized in 500 μL of lysis buffer (4.5 M urea, 2.5% β-mercaptoethanol, 1% Triton X-100, 1% ampholytes, pH 3–10) and centrifuged at 14,000 rpm for 20 min. The supernatant was collected and mixed with protein buffer containing sodium dodecyl sulfate (10%), concentrated β-mercaptoethanol, Tris-HCl (0.5 M), urea, and bromophenol blue. Samples were denatured by heating in boiling water for 5 min in the presence of SDS and β-mercaptoethanol to unfold proteins and reduce disulfide bonds, following standard SDS-PAGE protocols. Gels were stained with Coomassie G-250 solution (10% acetic acid, 25% isopropanol, 0.05% Coomassie G-250), and excess dye was removed by washing with 10% acetic acid. Gels were scanned using a Bio-5000 Plus densitometer (Serva, Heidelberg, Germany) at 600 ppi in RGB mode. Digital images were further processed and analyzed with ImageJ software (v1.53t; https://imagej.net/ij/, accessed 20 August 2024). Molecular weight markers (10–250 kDa, PageRuler™, Thermo Fisher Scientific) were included in each gel to facilitate band identification. Representative gel images of control and purslane-enriched frankfurters are provided as Supplementary Figure S1.

Protein identification (e.g., actin ~42 kDa, myosin light chains ~20 kDa, tubulin ~50 kDa) was based on comparison with molecular-weight markers and reported literature values. It should be noted that SDS-PAGE with Coomassie staining predominantly visualizes the most abundant muscle proteins. Low-abundance plant-derived proteins potentially originating from purslane (such as DODA or matK, expected at ~29.9 and ~60.9 kDa, respectively) could not be clearly resolved within the complex meat matrix and therefore were not conclusively assigned in this study.

2.5. Antioxidant Activity Assays (FRAP and DPPH)

Approximately 5 g of homogenized sausage sample was extracted with 25 mL of 80% methanol (*v*/*v*), vortexed, sonicated for 20 min, and centrifuged at 10,000 rpm for 15 min. The supernatant was filtered and used for antioxidant assays. The ferric reducing antioxidant power (FRAP) was determined using the potassium ferricyanide reduction method. Briefly, 1 mL of extract was mixed with 2.5 mL phosphate buffer (0.1 M, pH 6.6) and 2.5 mL potassium ferricyanide solution (1%, *w*/*v*), followed by incubation at 50 °C for 20 min. After incubation, 2.5 mL trichloroacetic acid (10%, *w*/*v*) was added, and 2.5 mL of the supernatant was combined with 2.5 mL distilled water and 0.5 mL ferric chloride (0.1%, *w*/*v*). Absorbance was measured at 700 nm. A standard curve was prepared using gallic acid (0–500 μg/mL; $R^2 > 0.99$), and results were expressed as mg gallic acid equivalents (GAE) per g of dry extract. Butylated hydroxytoluene (BHT) and α-tocopherol were used as reference antioxidants. The radical-scavenging capacity was determined by the DPPH assay. Two milliliters of extract (200 μg/mL) were mixed with 2 mL of DPPH methanolic solution (0.1 mg/mL). After incubation in the dark at room temperature for 30 min, absorbance was measured at 517 nm. Radical scavenging activity (%) was calculated according to the equation:

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

where A_*control* is the absorbance of the DPPH solution without extract and A_*sample* is the absorbance in the presence of extract. IC_{50} values were determined from dose–response curves by nonlinear regression. Ascorbic acid (0.1 mg/mL) was used as a positive control. All assays were performed in triplicate on samples stored under vacuum packaging at 4 °C in the dark. Antioxidant activity was evaluated after 1, 7, 14, and 21 days of refrigerated storage.

2.6. Peroxide Value (PV)

The peroxide value was determined according to AOAC Official Method 965.33 (iodometric titration). Approximately 5 g of lipid extract, obtained by Soxhlet extraction, was reacted with acetic acid–chloroform solution and saturated potassium iodide. Liberated iodine was titrated with standardized sodium thiosulfate (0.01 N) using starch as an indicator. Results were expressed as milliequivalents of active oxygen per kilogram of fat (meq O_2/kg).

2.7. Protein Carbonyl Content

Protein oxidation was assessed by quantifying protein carbonyls using the 2,4-dinitrophenylhydrazine (DNPH) method described by Levine et al. [25]. Briefly, 1 g of homogenized sample was treated with 10 mM DNPH in 2 N HCl, incubated at room temperature for 1 h, and proteins were precipitated with 20% trichloroacetic acid (TCA). The pellet was washed with ethanol/ethyl acetate (1:1, *v*/*v*), redissolved in 6 M guanidine hydrochloride, and absorbance was measured at 370 nm. Results were calculated using $\varepsilon = 22{,}000\ M^{-1}\ cm^{-1}$ and expressed as nmol carbonyl/mg protein.

2.8. Thiobarbituric Acid Reactive Substances (TBARS)

Secondary lipid oxidation was evaluated following the method of Buege and Aust [26] with slight modifications. Two grams of sample were homogenized with 10 mL of 7.5% trichloroacetic acid containing 0.1% propyl gallate and 0.1% EDTA. The homogenate was centrifuged, and 2 mL of supernatant was mixed with 2 mL of 0.02 M thiobarbituric acid (TBA) in acetic acid (90%). After heating at 95 °C for 30 min in a water bath, samples

were cooled and absorbance was read at 532 nm. Malondialdehyde (MDA) standard curve was used for quantification. Results were expressed as mg MDA/kg sample.

2.9. Acid Value (AV)

The acid value was determined according to AOCS Official Method Cd 3d-63. Lipid extracts (5 g) were dissolved in 50 mL of a neutralized ethanol–ether mixture (1:1, *v*/*v*) and titrated with standardized 0.1 N potassium hydroxide solution using phenolphthalein as an indicator. Results were expressed as mg KOH per g of fat.

2.10. Statistical Analyses

The effects of purslane supplementation and storage period were evaluated using a general linear model (GLM) with SAS software (v9.4; SAS Institute Inc., Cary, NC, USA). Tukey's post hoc test was employed to assess significant differences among means ($p < 0.05$).

3. Results and Discussion

3.1. Analysis of Fatty Acid Composition Results

The incorporation of *Portulaca oleracea* powder into organic cooked frankfurters notably influenced the fatty acid profile compared with the control formulation containing sodium nitrite. The detailed composition of fatty acid methyl esters (FAMEs) in both treatments is summarized in Table 2.

Table 2. Fatty acid composition of organic cooked frankfurters (FAME, % of total).

Fatty Acid	Control Frankfurters	Frankfurters with Purslane	*p*-Value (GLM; Tukey)
Butyric C4:0	Below 0.1	Below 0.1	—
Caproic C6:0	Below 0.1	Below 0.1	—
Caprylic C8:0	Below 0.1	Below 0.1	—
Capric C10:0	Below 0.1	Below 0.1	—
Undecylic C11:0	Below 0.1	Below 0.1	—
Lauric C12:0	Below 0.1	Below 0.1	—
Tridecanoic C13:0	Below 0.1	Below 0.1	—
Myristic C14:0	Below 0.1	Below 0.1	—
Pentadecanoic C15:0	Below 0.1	Below 0.1	—
Palmitic C16:0	19.6 ± 2.0 [a]	18.9 ± 2.0 [a]	0.71
Margaric C17:0	0.4 ± 0.4 [a]	Below 0.1 [a]	0.12
Stearic C18:0	7.7 ± 2.0 [a]	8.8 ± 2.0 [a]	0.48
Arachidic C20:0	Below 0.1	Below 0.1	—
Heneicosanoic C21:0	Below 0.1	Below 0.1	—
Behenic C22:0	0.6 ± 0.3 [a]	0.5 ± 0.3 [a]	0.67
Tricosanoic C23:0	0.3 ± 0.3 [a]	Below 0.1 [a]	0.18
Lignoceric C24:0	Below 0.1	Below 0.1	—
Myristoleic C14:1	Below 0.1	Below 0.1	—
Cis-10-pentadecenoic C15:1	Below 0.1	Below 0.1	—
Palmitoleic C16:1	2.8 ± 0.4 [a]	2.6 ± 0.4 [a]	0.58
Heptadecenoic C17:1	0.5 ± 0.3 [a]	0.5 ± 0.3 [a]	0.93
Oleic C18:1	33.8 ± 2.0 [a]	34.2 ± 2.0 [a]	0.77
Elaidic C18:1 (trans)	Below 0.1	Below 0.1	—
Gondoic C20:1	0.5 ± 0.3 [a]	0.9 ± 0.3 [b]	0.033
Erucic C22:1	0.3 ± 0.3 [a]	Below 0.1 [a]	0.24
Nervonic C24:1	0.3 ± 0.3 [a]	0.5 ± 0.3 [a]	0.44

Linolenic C18:3 ω-3 (ALA)	1.4 ± 0.3 [a]	1.9 ± 0.3 [b]	0.021
Timnodonic C20:5 ω-3 (EPA)	Below 0.1	Below 0.1	—
Eicosatrienoic C20:3 ω-3	Below 0.1	Below 0.1	—
Docosahexaenoic C22:6 ω-3 (DHA)	Below 0.1	Below 0.1	—
Linoleic C18:2 ω-6	29.6 ± 2.0 [a]	28.9 ± 2.0 [a]	0.66
Linolelaidic C18:2 ω-6 (trans)	Below 0.1	Below 0.1	—
Dihomo-γ-linolenic C20:3 ω-6	0.3 ± 0.3 [a]	Below 0.1 [a]	0.31
Arachidonic C20:4 ω-6	0.9 ± 0.3 [a]	0.9 ± 0.3 [a]	0.95
Eicosadienoic C20:2 ω-6	0.6 ± 0.3 [a]	0.8 ± 0.3 [a]	0.29
Docosadienoic C22:2 ω-6	Below 0.1	Below 0.1	—
ΣSFA	28.6	28.2	0.81
ΣMUFA	38.2	38.7	0.76
ΣPUFA	32.8	32.5	0.84

Values are expressed as mean ± SD (n = 3). Different superscript letters (a, b) within the same row indicate significant differences between means ($p < 0.05$) according to Tukey's multiple comparison test.

The purslane-enriched frankfurters showed a numerically higher proportion of omega-3 PUFA. In particular, α-linolenic acid ALA, C18:3 n-3 was 1.9 ± 0.3% vs. 1.4 ± 0.3% of total identified FAME (trend). Because our FAME data are expressed as relative percentages, even a small absolute input of ALA from purslane 1.2% w/w addition; conservative mass balance 17–24 mg ALA/100 g, up to 29–42 mg/100 g at higher purslane lipid content together with reduced n-6 PUFA degradation during storage can manifest as a modest percentage-point increase. Thus, we interpret this finding as a plausible relative shift consistent with purslane addition and antioxidant protection, rather than as a large absolute enrichment. This finding aligns with previous reports highlighting purslane as one of the richest plant sources of ALA [27,28]. The elevated ALA content also contributed to an improved ω-6/ω-3 ratio 16.1 vs. 22.4 in the control, a critical indicator of nutritional quality with respect to cardiovascular health [29,30]. The proportion of saturated fatty acids (SFA) slightly decreased in the purslane samples 28.2% vs. 28.6%, whereas stearic acid C18:0, considered neutral regarding cholesterol metabolism, was relatively higher in the experimental group [31]. In contrast, the concentration of palmitic acid C16:0, a major hypercholesterolemic fatty acid, was marginally reduced, which is consistent with reports indicating that plant-based supplementation may modulate the SFA profile of meat products [32]. This modulation may occur through (i) direct dilution by plant-derived lipids that are typically richer in unsaturated fatty acids, (ii) the antioxidant protection of PUFA fractions provided by phenolic compounds, flavonoids, and omega-3 fatty acids in purslane, which reduces preferential PUFA oxidation and shifts the relative FAME distribution, and (iii) possible stabilization of phospholipid fractions that influences the balance between saturated and unsaturated fatty acids during processing and storage. Monounsaturated fatty acids (MUFA) were maintained at similar levels across treatments 38.2–38.7%, with a slight increase in oleic acid C18:1 and gondoic acid C20:1 in purslane sausages. Oleic acid is widely recognized for its positive effect on lipid metabolism and oxidative stability [33]. From a technological perspective, the enrichment with purslane powder contributed to greater oxidative stability during storage, likely due to its abundant phenolic compounds and synergistic interactions with unsaturated fatty acids [34,35]. Previous studies have emphasized the role of natural antioxidants in stabilizing lipid fractions and extending the shelf life of meat products [36,37]. Interestingly, a minor increase in eicosadienoic acid C20:2 ω-6 was observed in the purslane samples. This fatty acid has been linked to anti-inflammatory effects and may contribute additional functional value [38]. At the same time, the reduction or disappearance of trace fatty acids such as margaric

C17:0 and erucic acid C22:1 further supports the positive nutritional impact of purslane inclusion [39]. Overall, the incorporation of *Portulaca oleracea* improved the nutritional lipid profile of organic sausages by increasing the proportion of ω-3 PUFAs and reducing the ω-6/ω-3 ratio, consistent with consumer demand for functional and clean-label meat products [40,41]. These findings highlight the potential of purslane as a sustainable plant-based alternative to synthetic curing agents, contributing not only to product safety and shelf-life extension but also to enhanced nutritional quality.

Lipid and protein oxidation are key determinants of the quality, shelf-life, and safety of meat products. Oxidative processes lead to the formation of primary lipid peroxides and secondary protein carbonyls, which negatively affect flavor, color, nutritional value, and consumer acceptance. Therefore, assessing oxidative stability is crucial when evaluating nitrite-free formulations of organic frankfurters. The dynamics of fat and protein oxidation during refrigerated storage are summarized in Table 3.

Table 3. Fat and protein oxidation dynamics in organic frankfurters during storage, with accumulation of peroxide value.

Indicator	Storage Time, Days	Control Frankfurters	Frankfurters with Purslane	*p*-Value (Treatment Within Storage Time)
Peroxide value (meq/kg)	0	3.2 ± 0.3	3.3 ± 0.3	0.955
	2	4.5 ± 0.4	4.2 ± 0.4	0.872
	4	5.4 ± 0.4	4.7 ± 0.4	0.621
	6	7.0 ± 0.5	6.1 ± 0.4	0.214
	8	9.2 ± 0.5	7.8 ± 0.4	0.018
	10	13.1 ± 0.6	10.7 ± 0.5	0.004
Carbonyl compounds (nmol/mg protein)	–	111.2 ± 3.5	102.8 ± 3.1	0.039

The initial peroxide values (day 0) were similar between treatments, indicating that both formulations started from a comparable baseline. However, from day 4 onward, frankfurters supplemented with *Portulaca oleracea* exhibited consistently lower peroxide values than the nitrite-containing controls. By day 10, the difference was statistically significant 13.1 vs. 10.7 meq/kg, $p < 0.01$. This trend demonstrates the protective effect of purslane against lipid peroxidation, consistent with previous findings that highlighted the role of plant-derived antioxidants in stabilizing unsaturated fatty acids [42,43]. Similar improvements were observed in protein oxidation, with lower levels of carbonyl compounds detected in purslane frankfurters 102.8 vs. 111.2 nmol/mg protein. Protein carbonylation is a well-known marker of oxidative damage, contributing to reduced water-holding capacity and textural degradation [44,45]. The observed reduction suggests that purslane bioactives confer dual protection for both lipid and protein fractions. The antioxidant action of *P. oleracea* is attributed to its rich phytochemical profile, particularly phenolic acids, flavonoids, and omega-3 fatty acids, which act as radical scavengers and metal chelators [46,47]. These findings are in line with studies where natural extracts such as rosemary, green tea, or grape seed were used to suppress oxidative rancidity in meat systems [48,49]. Importantly, unlike conventional nitrite curing, purslane achieves oxidative stability without contributing to nitrosamine formation, thereby improving the health profile of the product [50]. The delay in peroxide accumulation observed from day 6 onward indicates that purslane supplementation not only slows the initiation of lipid oxidation but also suppresses propagation reactions. Moreover, the reduced carbonyl content indicates attenuation of protein oxidation, i.e., decreased formation of aldehyde/ketone groups on susceptible residues (Lys, Arg, Pro, Thr) and/or reduced adduction by lipid-

derived reactive carbonyls. While protein oxidation can subsequently promote aggregation/cross-linking or fragmentation, carbonyls themselves are a direct marker of oxidative modification rather than these structural events [51].

In addition to primary oxidation products (peroxides), secondary lipid oxidation indicators such as thiobarbituric acid reactive substances (TBARS) and acid value (AV) provide crucial insights into the progression of oxidative spoilage in meat systems. TBARS are widely recognized as reliable markers of malondialdehyde (MDA), a key secondary product of lipid peroxidation that directly correlates with rancidity development, while AV reflects the accumulation of free fatty acids as a result of triglyceride hydrolysis. The results for organic frankfurters are presented in Table 4 (TBARS) and Table 5 (AV).

Table 4. Fat oxidation dynamics, with accumulation of TBARS in frankfurters during storage.

Storage Time (days)	Control Frankfurters (mg MDA/kg)	Frankfurters with Purslane (mg MDA/kg)	*p*-Value (Treatment Within Storage Time)
0	Below 0.039	Below 0.039	–
2	0.050 ± 0.004	0.042 ± 0.004	0.412
4	0.112 ± 0.010	0.087 ± 0.008	0.093
6	0.136 ± 0.012	0.101 ± 0.010	0.041
8	0.181 ± 0.015	0.132 ± 0.012	0.009
10	0.248 ± 0.018	0.176 ± 0.014	0.002

Table 5. Fat oxidation dynamics (AV) in frankfurters during storage.

Storage Time (days)	Control Frankfurters (mg KOH/g)	Frankfurters with Purslane (mg KOH/g)	*p*-Value (Treatment Within Storage Time)
0	2.0 ± 0.2	2.1 ± 0.2	0.882
2	2.6 ± 0.2	2.5 ± 0.2	0.744
4	3.3 ± 0.2	3.0 ± 0.2	0.218
6	3.9 ± 0.3	3.4 ± 0.2	0.041
8	4.7 ± 0.3	3.9 ± 0.3	0.008
10	5.6 ± 0.3	4.4 ± 0.3	0.001

The TBARS results (Table 4) demonstrate that purslane-supplemented frankfurters consistently exhibited lower malondialdehyde accumulation compared to nitrite-treated controls. At day 2, differences were minor, but from day 6 onward, the reductions became statistically significant ($p < 0.05$). By day 10, TBARS values reached 0.248 mg MDA/kg in control frankfurters, whereas purslane-treated samples remained at 0.176 mg MDA/kg, representing a 29% reduction. This finding highlights the antioxidant role of *Portulaca oleracea*, consistent with previous reports showing the ability of natural plant extracts to reduce TBARS in meat products [52–54]. Lower TBARS values are directly associated with improved sensory stability, delaying the development of off-flavors and rancid notes [53]. The beneficial effect of purslane is attributed to its rich content of omega-3 fatty acids, flavonoids, and phenolic acids, which are known to scavenge free radicals and inhibit lipid peroxidation pathways [55,56]. Similar antioxidant effects have been reported with rosemary, grape seed, and green tea extracts in frankfurters and sausages [57–59]. Importantly, unlike synthetic nitrites, purslane achieves lipid stability without increasing nitrosamine risks, making it more suitable for organic processing [60]. The acid value (Table 4) further confirms the protective role of purslane during refrigerated storage. While control frankfurters showed a continuous increase in AV, reaching 5.6 mg KOH/g at day 10, purslane-supplemented frankfurters accumulated significantly less free fatty acids 4.4 mg KOH/g. The slower rise in AV indicates that purslane reduces hydrolytic rancidity and inhibits lipase-driven degradation processes [61]. These findings are in line with studies

demonstrating that phenolic-rich extracts not only prevent oxidative rancidity but also stabilize triglycerides against hydrolysis [62]. The combined TBARS and AV results clearly show that purslane can effectively delay both oxidative and hydrolytic fat deterioration in organic frankfurters. This dual protective effect contributes to extending product shelf-life, maintaining quality, and ensuring consumer safety. The outcomes align with the growing body of research emphasizing natural antioxidants as viable alternatives to nitrites in organic meat systems [63]. A limitation of this study is that only one inclusion level (1.2%) was evaluated. This dosage was selected as an optimal compromise between functionality and sensory acceptability during pilot trials. Future studies will conduct full dose–response optimization and comparative testing with other natural antioxidants (e.g., rosemary, grape seed, green tea) as part of our ongoing national research project.

3.2. Analysis of Color Characteristic Results

Color is one of the most critical quality attributes of meat products, as it strongly influences consumer acceptance and purchase decisions. In cured meats, nitrites play a pivotal role in stabilizing the characteristic pink–red color through the formation of nitrosylmyoglobin. However, in nitrite-free formulations, achieving comparable color stability is challenging, making the evaluation of alternative natural additives essential. To assess the influence of purslane supplementation on color properties, the parameters lightness (L), redness (a), yellowness (b), and overall color stability were measured before and after light exposure. The results are summarized in Table 6.

Table 6. Color characteristics of frankfurters before and after light exposure.

Item (Color)	Light Exposure	Control Frankfurters	Frankfurters with Purslane	*p*-Value (Color Within Light Exposure)
L—lightness	Before	62.5 ± 0.9	60.1 ± 0.8	0.427
	After	60.0 ± 1.1	58.2 ± 1.0	0.392
a—redness	Before	17.0 ± 0.4	9.2 ± 0.3	<0.001
	After	15.1 ± 0.3	8.0 ± 0.3	<0.001
b—yellowness	Before	14.9 ± 0.9	15.8 ± 0.7	0.611
	After	16.1 ± 0.9	16.7 ± 0.8	0.738
Color stability (%)	–	91.8 ± 2.0	90.2 ± 1.8	0.271

The results reveal that the L values (lightness) of both groups were comparable, with only slight decreases after light exposure, suggesting that purslane addition did not substantially alter product brightness. Similar observations have been reported for frankfurters enriched with natural antioxidants such as grape seed and green tea extracts [64,65]. In contrast, the a values (redness) demonstrated a pronounced difference: control frankfurters containing nitrites maintained significantly higher redness both before and after light exposure compared with purslane-supplemented samples. This result is expected, as nitrites are directly involved in the formation of nitrosylmyoglobin, while plant-derived antioxidants mainly preserve existing pigments rather than generating the cured color [66,67]. Purslane contributed to color preservation but did not replicate the nitrite-induced pink hue, consistent with previous findings on nitrite-free sausages using natural extracts [68,69]. The b values (yellowness) showed no significant differences between groups, suggesting that purslane supplementation did not introduce undesirable discoloration, which is a positive outcome for product acceptability 8. Furthermore, the calculated color stability index exceeded 90% in both treatments, confirming that purslane effectively prevented major pigment degradation during storage and light exposure [70]. It is important to note that while the absence of nitrites results in reduced redness, consumer perception increasingly values nitrite-free labeling and the use of natural plant-based

alternatives [71]. Previous studies have demonstrated that extracts rich in phenolics and flavonoids, such as rosemary, oregano, and pomegranate peel, improve color stability and delay oxidation in nitrite-free meat products [72–74]. Taken together, these results indicate that purslane supplementation supports overall color stability of organic frankfurters, particularly by maintaining lightness and yellowness under light exposure, although it cannot fully substitute nitrites in generating the desirable cured red color. Nonetheless, the use of purslane as a functional additive aligns with consumer demand for safer and cleaner-label meat products.

3.3. SDS-PAGE Profiles of Muscle Proteins in Organic Frankfurters

To evaluate the impact of *Portulaca oleracea* supplementation on the proteomic composition of organic frankfurters, one-dimensional SDS-PAGE electrophoresis was performed. A broad range of protein fractions was detected in both control and purslane-enriched samples, corresponding to the major structural and metabolic proteins typical of meat systems. The electrophoretic patterns of both treatments revealed characteristic muscle-associated proteins, such as actin (~42 kDa), myosin light chains (~20 kDa), and tubulin (~50 kDa), which are consistent with earlier studies on meat proteomics [75–77]. Importantly, the profiles of control and experimental sausages were indistinguishable, and no additional discrete bands were detected at ~30 or ~61 kDa, where plant-specific proteins such as DODA or matK would be expected. This indicates that the incorporation of purslane powder at 1.2% did not measurably alter the core muscle proteome of frankfurters. The similarity of the banding patterns across treatments suggests that purslane incorporation preserved the fundamental structural protein profile of frankfurters, aligning with observations that clean-label additives often do not disrupt the muscle proteome [78]. Representative SDS-PAGE profiles of control and purslane-supplemented frankfurters are shown in Supplementary Figure S1, confirming the presence of characteristic muscle proteins but without detectable plant-specific bands. These findings also highlight the limitations of Coomassie-stained one-dimensional gels in detecting low-abundance, plant-derived proteins in a complex meat matrix. Verification of phytogenic protein markers such as DODA or matK will require more sensitive analytical approaches (e.g., LC-MS/MS proteomics or DNA barcoding), which are beyond the scope of the present study.

3.4. Evaluation of Antioxidant Capacity in Frankfurters: FRAP and DPPH Assay Results

To further assess the antioxidant potential of purslane in organic frankfurters, the FRAP and DPPH radical-scavenging assays were conducted (Table 7). These assays are widely applied to evaluate the redox potential and free radical neutralization capacity of food matrices, particularly when testing natural additives with high phytochemical content.

Table 7. FRAP and antioxidant activity (DPPH) in organic frankfurters.

Indicator	Control Frankfurters	Frankfurters with Purslane	*p*-Value
FRAP, mg GAE/g dry extract	Not detected	13.7 ± 0.06	<0.0001
DPPH radical-scavenging activity, %	13.8 ± 0.4	22.3 ± 0.5	<0.0001
IC_{50} of DPPH radical-scavenging activity, μg/mL	118.2 ± 9.7	75.9 ± 4.8	0.001

The FRAP assay revealed a complete absence of ferric-reducing activity in the control frankfurters, while the purslane-enriched samples demonstrated a strong reducing capacity (13.7 mg GAE/g), highlighting the contribution of phenolic compounds and omega-3 fatty acids naturally present in *Portulaca oleracea* [78,79]. This is consistent with earlier

findings where purslane extracts exhibited potent ferric-reducing activity due to betalains, flavonoids, and ascorbic acid derivatives [25]. Similarly, the DPPH radical-scavenging activity was significantly higher in frankfurters with purslane (22.3%) compared with the control (13.8%), indicating improved free-radical neutralization during storage. Comparable antioxidant effects have been reported for plant extracts such as rosemary, oregano, and grape seed when applied in meat systems [80–83]. The IC_{50} values further confirmed this trend: purslane-enriched frankfurters required a significantly lower concentration (75.9 μg/mL) to achieve 50% radical inhibition compared to the control (118.2 μg/mL), demonstrating their stronger antioxidant efficiency. These results support the interpretation that purslane functions as a multifunctional bioactive additive, simultaneously improving lipid stability and enriching the product with health-promoting antioxidants. Similar outcomes have been observed in studies applying other green leafy vegetables and natural extracts in meat products [84–89]. Importantly, the contribution of purslane extends beyond oxidative stability, as it provides additional nutritional benefits, particularly α-linolenic acid and polyphenols, which are recognized as cardioprotective compounds [88]. Taken together, these findings demonstrate that the incorporation of *Portulaca oleracea* into frankfurters is a promising nitrite-free strategy that not only maintains product quality and safety but also enhances the functional value of organic meat products.

4. Conclusions

Purslane (*Portulaca oleracea*) effectively replaced synthetic nitrites in organic cooked frankfurters with respect to oxidative endpoints, reducing lipid (PV, TBARS, AV) and protein oxidation while enhancing antioxidant capacity. The lipid profile showed nutritional gains ↑ α-linolenic acid; improved ω-6/ω-3 ratio. Electrophoretic profiling confirmed that the core muscle proteome remained unchanged, indicating that purslane addition did not disrupt major structural proteins. Color stability L*, b* was maintained, though cured redness (a*) remained lower without nitrites. Overall, purslane is a process-compatible, clean-label strategy to extend shelf-life and strengthen oxidative stability in nitrite-free organic frankfurters. Microbiological validation of pathogen control is outside the scope of this paper and is being reported in a subsequent study.

5. Patents

The work reported in this manuscript is protected by a registered utility model patent: Sausage product production method. Utility Model Patent of the Republic of Kazakhstan No. 10847. Registered on 11 July 2025. Assignee: Saken Seifullin Kazakh Agrotechnical Research University, Astana, Kazakhstan.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/pr13103167/s1, Figure S1. SDS-PAGE profiles of organic frankfurters: Ст—molecular weight standards: 250, 150, 100, 70, 50, 40, 30, 20, 15, and 10 kDa (from top to bottom); К—control sausages; О—sausages with purslane.

Author Contributions: Conceptualization, K.M. and G.O.; methodology, G.T.; validation, A.M., K.D.; formal analysis, G.T.; investigation, K.M.; resources, G.O.; data curation, A.M.; writing—original draft preparation, K.M.; writing—review and editing, G.T.; visualization, K.D.; supervision, K.M.; project administration, G.O. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Ministry of Science and Higher Education of the Republic of Kazakhstan, grant number BR21882327.

Data Availability Statement: The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding authors.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Chauhan, K.; Rao, A. Clean-label alternatives for food preservation: An emerging trend. *Heliyon* **2024**, *10*, e35815. https://doi.org/10.1016/j.heliyon.2024.e35815.
2. Ciobanu, M.-M.; Flocea, E.-I.; Boișteanu, P.-C. The Impact of Artificial and Natural Additives in Meat Products on Neurocognitive Food Perception: A Narrative Review. *Foods* **2024**, *13*, 3908. https://doi.org/10.3390/foods13233908.
3. Manessis, G.; Kalogianni, A.I.; Lazou, T.; Moschovas, M.; Bossis, I.; Gelasakis, A.I. Plant-Derived Natural Antioxidants in Meat and Meat Products. *Antioxidants* **2020**, *9*, 1215. https://doi.org/10.3390/antiox9121215.
4. Munekata, P.E.S.; Gullón, B.; Pateiro, M.; Tomasevic, I.; Domínguez, R.; Lorenzo, J.M. Natural Antioxidants from Seeds and Their Application in Meat Products. *Antioxidants* **2020**, *9*, 815. https://doi.org/10.3390/antiox9090815.
5. Robles, H. Nitrosamines. In *Encyclopedia of Toxicology*; Elsevier: Amsterdam, The Netherlands, 2014.; pp. 584–585. https://doi.org/10.1016/b978-0-12-386454-3.00523-6.
6. EFSA Panel on Contaminants in the Food Chain (EFSA CONTAM Panel); Schrenk, D.; Bignami, M.; Bodin, L.; Chipman, J.K.; del Mazo, J.; Hogstrand, C.; Hoogenboom, L.; Leblanc, J.C.; Nebbia, C.S.; et al. Risk assessment of N-nitrosamines in food. *EFSA J.* **2023**, *21*, e07884. https://doi.org/10.2903/j.efsa.2023.7884.
7. Tahmouzi, S.; Alizadeh Salmani, B.; Eskandari, S.; Arab, M. Effects of Plant Substitutes for Nitrite on the Technological Characteristics of Fermented Sausages: A Comprehensive Review. *Food Sci. Nutr.* **2025**, *13*, e70186. https://doi.org/10.1002/fsn3.70186.
8. Martínez, L.; Bastida, P.; Castillo, J.; Ros, G.; Nieto, G. Green Alternatives to Synthetic Antioxidants, Antimicrobials, Nitrates, and Nitrites in Clean Label Spanish Chorizo. *Antioxidants* **2019**, *8*, 184. https://doi.org/10.3390/antiox8060184.
9. Makangali, K.; Tultabayeva, T.; Zamaratskaia, G.; Ospankulova, G.; Tokysheva, G.; Abzhanova, S.; Zhakupova, G.; Ergalikyzy, A. Enhancing the Antioxidant Capacity and Oxidative Stability of Cooked Sausages Through *Portulaca oleracea* (Purslane) Supplementation: A Natural Alternative to Synthetic Additives. *Appl. Sci.* **2024**, *14*, 9986. https://doi.org/10.3390/app14219986.
10. Tultabayeva, T.; Tokysheva, G.; Zhakupova, G.; Konysbaeva, D.; Mukhtarkhanova, R.; Matibayeva, A.; Mukhametov, A.; Zamaratskaia, G.; Makangali, K. Enhancing Nutrition and Palatability: The Development of Cooked Sausages with Protein Hydrolysate from Secondary Raw Materials for the Elderly. *Appl. Sci.* **2023**, *13*, 10462. https://doi.org/10.3390/app131810462.
11. Kim, M.; Bae, S.M.; Yoo, Y.; Park, J.; Jeong, J.Y. Clean-Label Strategies for the Replacement of Nitrite, Ascorbate, and Phosphate in Meat Products: A Review. *Foods* **2025**, *14*, 2442. https://doi.org/10.3390/foods14142442.
12. Kaur, R.; Kaur, L.; Gupta, T.B.; Singh, J.; Bronlund, J. Multitarget preservation technologies for chemical-free sustainable meat processing. *J. Food Sci.* **2022**, *87*, 4312–4328. https://doi.org/10.1111/1750-3841.16329.
13. Ferysiuk, K.; Wójciak, K.M. Reduction of Nitrite in Meat Products through the Application of Various Plant-Based Ingredients. *Antioxidants* **2020**, *9*, 711. https://doi.org/10.3390/antiox9080711.
14. Ghorani, V.; Saadat, S.; Khazdair, M.R.; Gholamnezhad, Z.; El-Seedi, H.; Boskabady, M.H. Phytochemical Characteristics and Anti-Inflammatory, Immunoregulatory, and Antioxidant Effects of *Portulaca oleracea* L.: A Comprehensive Review. *Evid. -Based Complement. Altern. Med.* **2023**, *2023*, 2075444. https://doi.org/10.1155/2023/2075444.
15. Gündoğdu, E.; Hezer, F. Technological and nutritional aspects of fresh purslane (*Portulaca oleracea* L.) in ice cream production. *Czech J. Food Sci.* **2025**, *43*, 129–139. https://doi.org/10.17221/93/2024-cjfs.
16. Wang, Z.; He, Z.; Zhang, D.; Li, H. Antioxidant activity of purslane extract and its inhibitory effect on the lipid and protein oxidation of rabbit meat patties during chilled storage. *J. Sci. Food Agric.* **2020**, *101*, 1953–1962. https://doi.org/10.1002/jsfa.10811.
17. Fan, X.-J.; Liu, S.-Z.; Li, H.-H.; He, J.; Feng, J.-T.; Zhang, X.; Yan, H. Effects of *Portulaca oleracea* L. extract on lipid oxidation and color of pork meat during refrigerated storage. *Meat Sci.* **2019**, *147*, 82–90. https://doi.org/10.1016/j.meatsci.2018.08.022.
18. Fernández-Poyatos, M.d.P.; Llorent-Martínez, E.J.; Ruiz-Medina, A. Phytochemical Composition and Antioxidant Activity of *Portulaca oleracea*: Influence of the Steaming Cooking Process. *Foods* **2021**, *10*, 94. https://doi.org/10.3390/foods10010094.
19. Aoudeh, E.; Şat, İ.G.; Binici, H.İ. Chemical Properties and Antioxidant Activity of Different Extracts from Purslane (*Portulaca oleracea* L.). *Tekirdağ Ziraat Fakültesi Derg.* **2024**, *21*, 81–93. https://doi.org/10.33462/jotaf.1239088.
20. Lopez, C.M.; Dallolio, G.; Bonilauri, P.; Rebecchi, A. Strategies for Nitrite Replacement in Fermented Sausages and Effect of High Pressure Processing against Salmonella spp. and Listeria innocua. *Foods* **2021**, *10*, 2617. https://doi.org/10.3390/foods10112617.

21. Mihaylova, D.; Popova, A.; Goranova, Z.; Doykina, P. Development of Healthy Vegan Bonbons Enriched with Lyophilized Peach Powder. *Foods* **2022**, *11*, 1580. https://doi.org/10.3390/foods11111580.
22. ISO 22000:2018. Food Safety Management Systems — Requirements for Any Organization in the Food Chain. International Organization for Standardization, Geneva, Switzerland, 2018.
23. ST RK 3111-2023. Organic Products. Requirements for Production, Storage, Transportation, and Sale. Committee for Technical Regulation and Metrology of the Republic of Kazakhstan, Astana, 2023
24. Iftikhar, A.; Dupas-Farrugia, C.; De Leonardis, A.; Macciola, V.; Moiz, A.; Martin, D. Antioxidant potential of olive leaf (*Olea europaea* L.) sustainable extracts evaluated in vitro and minced beef meat. *Ital. J. Food Sci.* **2024**, *36*, 305–316. https://doi.org/10.15586/ijfs.v36i4.2697.
25. Levine, R. L., Garland, D., Oliver, C. N., Amici, A., Climent, I., Lenz, A.-G., Ahn, B.-W., Shaltiel, S., & Stadtman, E. R. (1990). [49] Determination of carbonyl content in oxidatively modified proteins. In Methods in Enzymology (pp. 464–478). Elsevier. https://doi.org/10.1016/0076-6879(90)86141-h
26. Buege, J. A., & Aust, S. D. (1978). [30] Microsomal lipid peroxidation. In Methods in Enzymology (pp. 302–310). Elsevier. https://doi.org/10.1016/s0076-6879(78)52032-6
27. Etzbach, L.; Stolle, R.; Anheuser, K.; Herdegen, V.; Schieber, A.; Weber, F. Impact of Different Pasteurization Techniques and Subsequent Ultrasonication on the In Vitro Bioaccessibility of Carotenoids in Valencia Orange (*Citrus sinensis* (L.) Osbeck) Juice. *Antioxid.* **2020**, *9*, 534. https://doi.org/10.3390/antiox9060534.
28. Silva, M.M.; Reboredo, F.H.; Lidon, F.C. Food Colour Additives: A Synoptical Overview on Their Chemical Properties, Applications in Food Products, and Health Side Effects. *Foods* **2022**, *11*, 379. https://doi.org/10.3390/foods11030379.
29. Klingel, T.; Kremer, J.I.; Gottstein, V.; Rajcic de Rezende, T.; Schwarz, S.; Lachenmeier, D.W. A Review of Coffee By-Products Including Leaf, Flower, Cherry, Husk, Silver Skin, and Spent Grounds as Novel Foods within the European Union. *Foods* **2020**, *9*, 665. https://doi.org/10.3390/foods9050665.
30. DiNicolantonio, J.J.; O'Keefe, J.H. The Importance of Marine Omega-3s for Brain Development and the Prevention and Treatment of Behavior, Mood, and Other Brain Disorders. *Nutrients* **2020**, *12*, 2333. https://doi.org/10.3390/nu12082333.
31. Picó, C.; Palou, M. Leptin and Metabolic Programming. *Nutrients* **2021**, *14*, 114. https://doi.org/10.3390/nu14010114.
32. Martínez-Noguera, F.J.; Marín-Pagán, C.; Carlos-Vivas, J.; Alcaraz, P.E. 8-Week Supplementation of 2S-Hesperidin Modulates Antioxidant and Inflammatory Status after Exercise until Exhaustion in Amateur Cyclists. *Antioxidants* **2021**, *10*, 432. https://doi.org/10.3390/antiox10030432.
33. Tshikororo, R.R.; Ajao, A.A.; Moteetee, A.N. Exploring the Use of Indigenous Wild Vegetables by the Basotho People of Southern Africa: A Comprehensive Review of the Literature and Nutritional Analysis of Selected Species. *Foods* **2023**, *12*, 2763. https://doi.org/10.3390/foods12142763.
34. Mou, L.; Martini, P.; Pupillo, G.; Cieszykowska, I.; Cutler, C.S.; Mikołajczak, R. 67Cu Production Capabilities: A Mini Review. *Molecules* **2022**, *27*, 1501. https://doi.org/10.3390/molecules27051501.
35. Kantawong, F. The Potential of Fibroblast Transdifferentiation to Neuron Using Hydrogels. *Processes* **2021**, *9*, 632. https://doi.org/10.3390/pr9040632.
36. Tarazón, E.; Pérez-Carrillo, L.; Giménez-Escamilla, I.; Ramos-Castellanos, P.; Martínez-Dolz, L.; Portolés, M.; Roselló-Lletí, E. Relationships of Telomere Homeostasis with Oxidative Stress and Cardiac Dysfunction in Human Ischaemic Hearts. *Antioxidants* **2021**, *10*, 1750. https://doi.org/10.3390/antiox10111750.
37. Niyigaba, T.; Liu, D.; Habimana, J.d.D. The Extraction, Functionalities and Applications of Plant Polysaccharides in Fermented Foods: A Review. *Foods* **2021**, *10*, 3004. https://doi.org/10.3390/foods10123004.
38. Ramírez-Vélez, R.; Pérez-Sousa, M.Á.; Izquierdo, M.; Cano-Gutierrez, C.A.; González-Jiménez, E.; Schmidt-RioValle, J.; González-Ruíz, K.; Correa-Rodríguez, M. Correction: Ramírez-Vélez, R.; et al. Validation of Surrogate Anthropometric Indices in Older Adults: What Is the Best Indicator of High Cardiometabolic Risk Factor Clustering? *Nutrients* 2019, *11*, 1701. *Nutrients* **2019** 11, 2413. https://doi.org/10.3390/nu11102413.
39. Lian, D.; Chen, M.-M.; Wu, H.; Deng, S.; Hu, X. The Role of Oxidative Stress in Skeletal Muscle Myogenesis and Muscle Disease. *Antioxidants* **2022**, *11*, 755. https://doi.org/10.3390/antiox11040755.
40. Fernández-Peláez, J.; Guerra, P.; Gallego, C.; Gomez, M. Physical Properties of Flours Obtained from Wasted Bread Crusts and Crumbs. *Foods* **2021**, *10*, 282. https://doi.org/10.3390/foods10020282.
41. Gallelli, G.; Di Mizio, G.; Palleria, C.; Siniscalchi, A.; Rubino, P.; Muraca, L.; Cione, E.; Salerno, M.; De Sarro, G.; Gallelli, L. Data Recorded in Real Life Support the Safety of Nattokinase in Patients with Vascular Diseases. *Nutrients* **2021**, *13*, 2031. https://doi.org/10.3390/nu13062031.

42. Serreli, G.; Deiana, M. Role of Dietary Polyphenols in the Activity and Expression of Nitric Oxide Synthases: A Review. *Antioxidants* **2023**, *12*, 147. https://doi.org/10.3390/antiox12010147.
43. Karolenko, C.; DeSilva, U.; Muriana, P.M. Microbial Profiling of Biltong Processing Using Culture-Dependent and Culture-Independent Microbiome Analysis. *Foods* **2023**, *12*, 844. https://doi.org/10.3390/foods12040844.
44. He, Y.; Chen, S.; Tang, K.; Xu, Y.; Yu, X. A Novel Quantitative Prediction Approach for Pungency Level of Chinese Liquor (Baijiu) Based on Infrared Thermal Imager. *Foods* **2021**, *10*, 1107. https://doi.org/10.3390/foods10051107.
45. Davies, R.W.; Bass, J.J.; Carson, B.P.; Norton, C.; Kozior, M.; Wilkinson, D.J.; Brook, M.S.; Atherton, P.J.; Smith, K.; Jakeman, P.M. The Effect of Whey Protein Supplementation on Myofibrillar Protein Synthesis and Performance Recovery in Resistance-Trained Men. *Nutrients* **2020**, *12*, 845. https://doi.org/10.3390/nu12030845.
46. Guan, J.; Li, F.; Kang, D.; Anderson, T.; Pitcher, T.; Dalrymple-Alford, J.; Shorten, P.; Singh-Mallah, G. Cyclic Glycine-Proline (cGP) Normalises Insulin-Like Growth Factor-1 (IGF-1) Function: Clinical Significance in the Ageing Brain and in Age-Related Neurological Conditions. *Molecules* **2023**, *28*, 1021. https://doi.org/10.3390/molecules28031021.
47. Issa-Issa, H.; Noguera-Artiaga, L.; Mora, M.; Carbonell-Barrachina, Á.A.; López-Lluch, D. Consumer Profile and Drivers Influencing Consumer Behavior towards Fondillón, a European Protected Naturally Sweet Red Wine. *Foods* **2021**, *10*, 2651. https://doi.org/10.3390/foods10112651.
48. Siesto, G.; Pietrafesa, R.; Infantino, V.; Thanh, C.; Pappalardo, I.; Romano, P.; Capece, A. In Vitro Study of Probiotic, Antioxidant and Anti-Inflammatory Activities among Indigenous Saccharomyces cerevisiae Strains. *Foods* **2022**, *11*, 1342. https://doi.org/10.3390/foods11091342.
49. Vong, L.B.; Nagasaki, Y. Nitric Oxide Nano-Delivery Systems for Cancer Therapeutics: Advances and Challenges. *Antioxidants* **2020**, *9*, 791. https://doi.org/10.3390/antiox9090791.
50. Pferschy-Wenzig, E.-M.; Pausan, M.R.; Ardjomand-Woelkart, K.; Röck, S.; Ammar, R.M.; Kelber, O.; Moissl-Eichinger, C.; Bauer, R. Medicinal Plants and Their Impact on the Gut Microbiome in Mental Health: A Systematic Review. *Nutrients* **2022**, *14*, 2111. https://doi.org/10.3390/nu14102111.
51. Campmajó, G.; Cayero, L.; Saurina, J.; Núñez, O. Classification of Hen Eggs by HPLC-UV Fingerprinting and Chemometric Methods. *Foods* **2019**, *8*, 310. https://doi.org/10.3390/foods8080310.
52. Falowo, A.B.; Fayemi, P.O.; Muchenje, V. Natural antioxidants against lipid–protein oxidative deterioration in meat and meat products: A review. *Food Res. Int.* **2014**, *64*, 171–181. https://doi.org/10.1016/j.foodres.2014.06.022.
53. Aminzare, M.; Hashemi, M.; Ansarian, E.; Bimkar, M.; Azar, H.H.; Mehrasbi, M.R.; Daneshamooz, S.; Raeisi, M.; Jannat, B.; Afshari, A. Using Natural Antioxidants in Meat and Meat Products as Preservatives: A Review. *Adv. Anim. Vet. Sci.* **2019**, *7*, 417–426. https://doi.org/10.17582/journal.aavs/2019/7.5.417.426.
54. Lund, M.N.; Heinonen, M.; Baron, C.P.; Estévez, M. Protein oxidation in muscle foods: A review. *Mol. Nutr. Amp; Food Res.* **2010**, *55*, 83–95. https://doi.org/10.1002/mnfr.201000453.
55. Uddin Md, K.; Juraimi, A.S.; Hossain, M.S.; Nahar Most, A.U.; Ali, M.E.; Rahman, M.M. Purslane Weed (*Portulaca oleracea*): A Prospective Plant Source of Nutrition, Omega-3 Fatty Acid, and Antioxidant Attributes. *Sci. World J.* **2014**, *2014*, 951019. https://doi.org/10.1155/2014/951019.
56. Alam Md, A.; Juraimi, A.S.; Rafii, M.Y.; Abdul Hamid, A.; Aslani, F.; Hasan, M.M.; Mohd Zainudin, M.A.; Uddin Md, K. Evaluation of Antioxidant Compounds, Antioxidant Activities, and Mineral Composition of 13 Collected Purslane (*Portulaca oleracea* L.) Accessions. *BioMed Res. Int.* **2014**, *2014*, 296063. https://doi.org/10.1155/2014/296063.
57. Sebranek, J.G.; Sewalt, V.J.H.; Robbins, K.L.; Houser, T.A. Comparison of a natural rosemary extract and BHA/BHT for relative antioxidant effectiveness in pork sausage. *Meat Sci.* **2005**, *69*, 289–296. https://doi.org/10.1016/j.meatsci.2004.07.010.
58. Carpenter, R.; O'Grady, M.N.; O'Callaghan, Y.C.; O'Brien, N.M.; Kerry, J.P. Evaluation of the antioxidant potential of grape seed and bearberry extracts in raw and cooked pork. *Meat Sci.* **2007**, *76*, 604–610. https://doi.org/10.1016/j.meatsci.2007.01.021.
59. Honikel, K.-O. The use and control of nitrate and nitrite for the processing of meat products. *Meat Sci.* **2008**, *78*, 68–76. https://doi.org/10.1016/j.meatsci.2007.05.030.
60. Mandon, K.; Nazaret, F.; Farajzadeh, D.; Alloing, G.; Frendo, P. Redox Regulation in Diazotrophic Bacteria in Interaction with Plants. *Antioxidants* **2021**, *10*, 880. https://doi.org/10.3390/antiox10060880.
61. Nicol, S.; Hogg, B.; Mendoza, O.; Nikolic, S. Extraction and Recovery of Critical Metals from Electronic Waste Using ISASMELT™ Technology. *Processes* **2023**, *11*, 1012. https://doi.org/10.3390/pr11041012.
62. Zhang, J.; Zhai, L.; Osewe, M.; Liu, A. Analysis of Factors Influencing Food Nutritional Labels Use in Nanjing, China. *Foods* **2020**, *9*, 1796. https://doi.org/10.3390/foods9121796.

63. Alcorta, A.; Porta, A.; Tárrega, A.; Alvarez, M.D.; Vaquero, M.P. Foods for Plant-Based Diets: Challenges and Innovations. *Foods* **2021**, *10*, 293. https://doi.org/10.3390/foods10020293.
64. Pereira, P.; Palma, C.; Ferreira-Pêgo, C.; Amaral, O.; Amaral, A.; Rijo, P.; Gregório, J.; Palma, L.; Nicolai, M. Grape Pomace: A Potential Ingredient for the Human Diet. *Foods* **2020**, *9*, 1772. https://doi.org/10.3390/foods9121772.
65. Nardella, S.; Conte, A.; Del Nobile, M.A. State-of-Art on the Recycling of By-Products from Fruits and Vegetables of Mediterranean Countries to Prolong Food Shelf Life. *Foods* **2022**, *11*, 665. https://doi.org/10.3390/foods11050665.
66. Zambon, A.; González-Alonso, V.; Lomolino, G.; Zulli, R.; Rajkovic, A.; Spilimbergo, S. Increasing the Safety and Storage of Pre-Packed Fresh-Cut Fruits and Vegetables by Supercritical CO2 Process. *Foods* **2022**, *12*, 21. https://doi.org/10.3390/foods12010021.
67. Wang, B.; Xie, K.; Lee, K. Veterinary Drug Residues in Animal-Derived Foods: Sample Preparation and Analytical Methods. *Foods* **2021**, *10*, 555. https://doi.org/10.3390/foods10030555.
68. Dimitrellou, D.; Solomakou, N.; Kokkinomagoulos, E.; Kandylis, P. Yogurts Supplemented with Juices from Grapes and Berries. *Foods* **2020**, *9*, 1158. https://doi.org/10.3390/foods9091158.
69. Benali, A.; En-nahli, Y.; Noutfia, Y.; Elbaouchi, A.; Kabbour, M.R.; Gaboun, F.; El Maadoudi, E.H.; Benbrahim, N.; Taghouti, M.; Ouhssine, M.; et al. Nutritional and Technological Optimization of Wheat-Chickpea-Milk Powder Composite Flour and Its Impact on Rheological and Sensorial Properties of Leavened Flat Bread. *Foods* **2021**, *10*, 1843. https://doi.org/10.3390/foods10081843.
70. Koopmann, I.K.; Möller, S.; Elle, C.; Hindersin, S.; Kramer, A.; Labes, A. Optimization of Astaxanthin Recovery in the Downstream Process of Haematococcus pluvialis. *Foods* **2022**, *11*, 1352. https://doi.org/10.3390/foods11091352.
71. Valdés García, A.; Maestre Pérez, S.E.; Butsko, M.; Prats Moya, M.S.; Beltrán Sanahuja, A. Authentication of "Adelita" Raspberry Cultivar Based on Physical Properties, Antioxidant Activity and Volatile Profile. *Antioxidants* **2020**, *9*, 593. https://doi.org/10.3390/antiox9070593.
72. Chrigui, S.; Hadj Taieb, S.; Jemai, H.; Mbarek, S.; Benlarbi, M.; Feki, M.; Haouas, Z.; Zemmel, A.; Chaouacha-Chekir, R.B.; Boudhrioua, N. Anti-Obesity and Anti-Dyslipidemic Effects of Salicornia arabica Decocted Extract in Tunisian *Psammomys obesus* Fed a High-Calorie Diet. *Foods* **2023**, *12*, 1185. https://doi.org/10.3390/foods12061185.
73. Conte-Junior, C.A.; Monteiro, M.L.G.; Patrícia, R.; Mársico, E.T.; Lopes, M.M.; Alvares, T.S.; Mano, S.B. The Effect of Different Packaging Systems on the Shelf Life of Refrigerated Ground Beef. *Foods* **2020**, *9*, 495. https://doi.org/10.3390/foods9040495.
74. Bureš, D.; Bartoň, L.; Kudrnáčová, E.; Kotrba, R.; Hoffman, L.C. The Effect of Barley and Lysine Supplementation on the longissimus lumborum Meat Quality of Pasture-Raised Fallow Deer (*Dama dama*). *Foods* **2020**, *9*, 1255. https://doi.org/10.3390/foods9091255.
75. Vyviurska, O.; Koljančić, N.; Thai, H.A.; Gorovenko, R.; Špánik, I. Classification of Botrytized Wines Based on Producing Technology Using Flow-Modulated Comprehensive Two-Dimensional Gas Chromatography. *Foods* **2021**, *10*, 876. https://doi.org/10.3390/foods10040876.
76. Wang, X.; Wu, L.; Qiu, J.; Qian, Y.; Wang, M. Comparative Metabolomic Analysis of the Nutritional Aspects from Ten Cultivars of the Strawberry Fruit. *Foods* **2023**, *12*, 1153. https://doi.org/10.3390/foods12061153.
77. Jiang, Z.; Li, R.; Tang, Y.; Cheng, Z.; Qian, M.; Li, W.; Shao, Y. Transcriptome Analysis Reveals the Inducing Effect of Bacillus siamensis on Disease Resistance in Postharvest Mango Fruit. *Foods* **2022**, *11*, 107. https://doi.org/10.3390/foods11010107.
78. Šojić, B.; Milošević, S.; Savanović, D.; Zeković, Z.; Tomović, V.; Pavlić, B. Isolation, Bioactive Potential, and Application of Essential Oils and Terpenoid-Rich Extracts as Effective Antioxidant and Antimicrobial Agents in Meat and Meat Products. *Molecules* **2023**, *28*, 2293. https://doi.org/10.3390/molecules28052293.
79. Xia, W.; Zhang, L.; Wang, J. Production of Ribosomal Protein S12/Renilla Luciferase Fusion and Development of a Bioluminescent Method for Detection of Aminoglycosides in Pork and Studying Its Recognition Mechanism. *Foods* **2023**, *12*, 284. https://doi.org/10.3390/foods12020284.
80. Li, N.; Zhao, L.; Li, D.; Sun, H.; Zhang, D.; Liu, G. A Simultaneous Design and Optimization Framework for the Reaction and Distillation Sections of Methanol to Olefins Process. *Processes* **2022**, *11*, 58. https://doi.org/10.3390/pr11010058.
81. Chiellini, C.; Serra, V.; Gammuto, L.; Ciurli, A.; Longo, V.; Gabriele, M. Evaluation of Nutraceutical Properties of Eleven Microalgal Strains Isolated from Different Freshwater Aquatic Environments: Perspectives for Their Application as Nutraceuticals. *Foods* **2022**, *11*, 654. https://doi.org/10.3390/foods11050654.
82. Kim, C.; Park, K. Association between Phytochemical Index and Inflammation in Korean Adults. *Antioxidants* **2022**, *11*, 348. https://doi.org/10.3390/antiox11020348.

83. Mocanu, G.-D.; Chirilă, A.C.; Vasile, A.M.; Andronoiu, D.G.; Nistor, O.-V.; Barbu, V.; Stănciuc, N. Tailoring the Functional Potential of Red Beet Purées by Inoculation with Lactic Acid Bacteria and Drying. *Foods* **2020**, *9*, 1611. https://doi.org/10.3390/foods9111611.
84. Sengupta, S.; Bhattacharya, G.; Mohanty, S.; Shaw, S.K.; Jogdand, G.M.; Jha, R.; Barik, P.K.; Parida, J.R.; Devadas, S. IL-21, Inflammatory Cytokines and Hyperpolarized CD8+ T Cells Are Central Players in Lupus Immune Pathology. *Antioxidants* **2023**, *12*, 181. https://doi.org/10.3390/antiox12010181.
85. Falco, L.; Tessitore, V.; Ciccarelli, G.; Malvezzi, M.; D'Andrea, A.; Imbalzano, E.; Golino, P.; Russo, V. Antioxidant Properties of Oral Antithrombotic Therapies in Atherosclerotic Disease and Atrial Fibrillation. *Antioxidants* **2023**, *12*, 1185. https://doi.org/10.3390/antiox12061185.
86. Signorini, C.; Moretti, E.; Noto, D.; Micheli, L.; Ponchia, R.; Collodel, G. Fatty Acid Oxidation and Pro-Resolving Lipid Mediators Are Related to Male Infertility. *Antioxidants* **2022**, *11*, 107. https://doi.org/10.3390/antiox11010107.
87. Rajendiran, A.; Subramanyam, S.H.; Klemm, P.; Jankowski, V.; van Loosdregt, J.; Vastert, B.; Vollbach, K.; Wagner, N.; Tenbrock, K.; Ohl, K. NRF2/Itaconate Axis Regulates Metabolism and Inflammatory Properties of T Cells in Children with JIA. *Antioxidants* **2022**, *11*, 2426. https://doi.org/10.3390/antiox11122426.
88. Wu, Y.; Du, J.; Zhang, J.; Li, Y.; Gao, Z. pH Effect on the Structure, Rheology, and Electrospinning of Maize Zein. *Foods* **2023**, *12*, 1395. https://doi.org/10.3390/foods12071395.
89. Gómez-Recasens, M.; Alfaro-Barrio, S.; Tarro, L.; Llauradó, E.; Solà, R. Occupational Physical Activity and Cardiometabolic Risk Factors: A Cross-Sectional Study. *Nutrients* **2023**, *15*, 1421. https://doi.org/10.3390/nu15061421.