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Article

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CHARD POWDER AS NATURAL SOURCE OF NITRITES FOR FERMENTED DRIED SAUSAGES: PHYSICOCHEMICAL AND MICROBIOLOGICAL STUDIES

Nitrites are added to sausage products to accelerate ripening, improve color and microbiological indicators. Chard (Beta vulgaris var. cicla) is one of the best natural sources of nitrites, as it contains a lot of nitrates – 1680 mg/kg in a fresh vegetable. However, chard has not been used as a source of nitrites in sausage technology and in cooking it is used only in fresh, boiled or sautéed form. There are no technologies for industrial drying and processing of chard into powder. Chard powder was obtained by microwave vacuum drying and grinding to 200 microns, which was determined by the appropriate sieve size. Five samples of fermented dried sausages were prepared and evaluated during the ripening process: C1 (without nitrite and sodium nitrate), C2 (100 mg/kg sodium nitrite and 100 mg/kg sodium nitrate), M1 (0.5 % chard powder), M2 (1 % chard powder) and M3 (1.5 % chard powder). With the addition of 1.5 % chard powder (Sample M3), the protein content increased by 22.74 %, ash content increased by 41.82 %, and dietary fiber content increased by 93.75 % compared to the control sample C2. Nitrite was formed from chard powder during the ripening process, especially in treatments M2 and M3. After the entire production process of fermented dried sausages for 35 days, nitrates were detected only in sample C2. The yield of fermented dried sausages enriched with chard powder decreased by 15.95 % compared to the control sample C2. Chard powder improved the microbiological parameters of sausage products, especially in sample M3. The content of aerobic mesophilic bacteria increased by 5.84 % and the content of lactic acid bacteria increased by 8.96 % in sample M3 compared to control C2 after 35 days of fermentation and drying, which is related to the activity of the starter. The organoleptic parameters of fermented dried sausages enriched with chard powder for samples M2 and M3 were better in terms of color and texture. The results of the analysis of nutritional value, pH, nitrites and nitrates indicate the effectiveness of adding 1.5 % chard powder (Sample M3) obtained by microwave vacuum drying as a potential source of nitrites in fermented dried sausages.

Keywords: *chard powder, drying, nitrate-rich beets, vegetables, food coloring, minced meat products, quality characteristics.*

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1. Introduction

Meat products contain proteins of high biological value and important trace elements, such as a complex of B vitamins and minerals [1, 2]. The high content of fat, sodium and some additives in meat products is an important problem for a healthy diet [3]. Innovative research in the field of improving recipes of meat products is aimed at partial or complete replacement of meat with vegetable protein isolate, which has the functional and technological properties of raw materials of animal origin [4, 5]. Sodium nitrite is an additive that performs many functions in meat products, such as inhibiting microorganisms, especially *Clostridium botulinum* [6], stabilizing the color and flavor of cured meat products, but it has received many criticisms in meat products [7–9]. Sodium nitrite is a powerful antioxidant that can slow down lipid oxidation, the formation of a stable complex with Fe(II) is the main mechanism to prevent the formation of Fe(III), a powerful oxidation catalyst [10]. However, the use of sodium nitrite is controversial in the scientific community and in health authorities mainly because there is no clear evidence of a relationship between nitrite consumption and the formation of nitrosamines [11, 12]. Formation of nitrosamines was observed at initial nitrite content above 120 mg/kg and high temperature (>120 °C) in cooked meat products [13]. In addition, the formation of nitrosamines by dissociation of nitrous acid and secondary amines is accelerated at pH 3.5 [14, 15]. In this regard, the control of the initial content of nitrites and residual nitrites in

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meat products is important, and the use of nitrites in the food industry is strictly regulated, but its reduction or replacement is still a problem [16]. Given the growing health concerns over nitrosamine consumption and clean label claims, reformulation of meat products is becoming increasingly necessary. There are many advanced studies on the creation of new recipes of sausage products based on natural plant raw materials, which create an impetus for the future development of the food industry [17]. Some studies have evaluated potential natural substitutes for sodium nitrite to create healthy meat product formulations while maintaining sensory properties and microbiological safety [18–21].

An interesting strategy to replace sodium nitrite is to combine nitrate-rich vegetables with starter cultures that convert nitrates to nitrites [22]. The search for new raw materials specific to a certain region of the country to reduce the cost and improve the health of the population is a common topic for research [23]. Local raw materials can be effectively grown by farmers on personal farms, which will improve the economy and sustainability of the community [24]. One of the most used microorganisms for fermentation of sausage products is *Staphylococcus carnosus*, which improve the taste properties of dried meat [25–27].

Restaurant farms mostly use local raw materials to improve economic indicators, and fermented dried sausages, which are traditional for Ukraine, are better made from meat, hydrobionts and vegetables of regional origin [28]. Spinach, lettuce, and celery are used as nitrate-rich plant sources in natural cured meat products [29–32]. In addition, radish and beet have great potential in the production of fermented meat products due to their high nitrate content, in addition to promoting color development in meat products, depending on the level used in the formulation [33, 34]. Similarly, beetroot (*Beta vulgaris*) is a vegetable that also contains a high content of nitrates. Beets, which belong to the *Chenopodiaceae* family, are rich in phenolic acids, vitamins and have antioxidant capacity. In addition, beetroot can be used as a natural dye due to its water-soluble pigments known as betalains [35, 36]. Betalains are effectively used in the technology of traditional Ukrainian cooked sausages to provide a constant color to sausage products, regardless of the features of the recipe and raw materials [37].

The aim of research is to investigate the effectiveness of using chard powder as a natural source of nitrites in fermented dried sausages, to evaluate the effect of chard powder during ripening and storage using physicochemical and microbiological analyses.

2. Materials and Methods

Materials and raw materials for research. Chard (*Beta vulgaris var. cicla*) variety «Burpee's Rhubarb Chard» of the 2023 harvest was purchased from a farm in Sumy, Ukraine. Other components of the fermented sausage recipe were purchased at the local market in Sumy, Ukraine. All reagents used in the experiment were of analytical quality.

Preparation of chard powder. Fresh chard (*Beta vulgaris var. cicla*) is washed, chopped, evenly placed on trays and placed in a microwave vacuum dryer. The drying process was carried out at a microwave power of 500 W and a vacuum degree of 0.08 MPa. After drying for 60 min, the microwave power was switched to 350 W to continue drying. The drying process was stopped when the final moisture content of the chard pieces was less than 8.5 %. The chard pieces were crushed, passed through a 200 µm sieve, the chard powder was packed in a plastic bag and stored at 25 °C for further use.

Determination of the content of moisture, ash, lipids, pH, nitrates and nitrites in chard powder. The content of moisture, protein and ash in radish and beet powders was determined according to AOAC (Association of Official Agricultural Chemists) [38]. Quantification of lipids was carried out according to the methodology of the rapid method of complete extraction and purification of lipids [39]. The pH was measured in a solution obtained by mixing 1 g of radish or beetroot powder in 9 g of water using a calibrated pH meter. Nitrate content was determined by reducing it to nitrite with a cadmium column. N-(1-naphthyl) ethylenediamine dihydrochloride and sulfonamide were added to the nitrite. The absorbance of the solutions was measured at 540 nm. All determinations were performed three times.

Production of fermented dried sausages. The production technology of fermented dried sausages enriched with chard powder involves grinding pork, pork fat and garlic to a uniform consistency on a meat grinder (nozzle d=10 mm). Sodium chloride was added to minced meat to extract myofibrillar proteins. Minced meat was mixed with other components of the recipe to a homogeneous consistency and filled into edible casings (d=50 mm) to obtain 300 g of fermented sausages. Fermented sausages were kept in the fermentation chamber at the specified temperature and air humidity: 1st day 25 °C/95 %; 4th day 22 °C/89 %; 8th day 18 °C/85 %; up to 35 days 15 °C/75 % at air speed ≤0.35 m/s. Fermented dried sausages were vacuum packed and stored at 5 °C for 60 days.

Physico-chemical analyzes and yield of fermented dried sausages. The content of moisture, protein and ash in chard powder was determined according to AOAC [38]. Quantification of lipids was carried out according to the methodology of the rapid method of complete extraction and purification of lipids [39]. The pH was measured in a solution obtained by mixing 1 g of chard powder in 9 g of water using a calibrated pH meter. The yield (%) was determined in 10 units/sample. Sausages were weighed during processing on days 0 and 35. Weight loss (%) was determined by the equation: (weight of product on the day of selection during fermentation – weight on day 0 of fermentation)/weight of product on the day of selection during fermentation×100. Determination of residual nitrite was based on the AOAC method [40]. Determination of nitrates was carried out using a cadmium column, and absorbance was measured at 540 nm. Determination of nitrites and nitrates was carried out during fermentation (0 and 35 days) in three replicates for each cycle.

Microbiological research of sausage products. Microbiological analyzes of aerobic mesophilic bacteria and total coliforms were carried out according to the collection of methods of microbiological research of food products [41]. Lactic acid bacteria were quantified according to ISO 15214 [42]. Two parts of each cycle (n=6) were used, and determinations were made during treatment (0 and 35 days).

Organoleptic characteristics. Ten experts evaluated sausage products using the Score Card method to assess sensory parameters, namely: color, consistency, aroma, taste, and overall acceptability [43]. The obtained values from the participants were evaluated using a one-way analysis of variance and expressed as a mean value.

Statistical analysis. The results of the studies were expressed in the form of an average value with the number of experiments n and a standard error of $p<0.05$.

3. Results and Discussion

Research on chard powder (Beta vulgaris var. cicla). Chard (*Beta vulgaris var. cicla*) is one of the best natural sources of nitrites, as it contains a lot of nitrates – 1680 mg/kg in a fresh vegetable. chard is a source of nitrates, minerals, dietary fiber and protein. After microwave vacuum drying and significant reduction of moisture by 13 times in chard, the concentration of nitrates, minerals, dietary fiber and protein increased significantly, which is shown in Table 1. Chard powder showed a nitrate content of 21.600 mg/kg after the drying process. It should be noted that chard powder is classified as high in nitrates, as it contains more than 2500 mg/kg of nitrates [44]. The mineral content of 19.61 g/100 g, dietary fiber 20.91 g/100 g and protein 28.76 g/100 g were at a high level, which is good for a sausage formulation.

Table 1

Physicochemical analysis of chard powder (*Beta vulgaris var. cicla*) $n=3$

Parameters, %	Chard powder (*Beta vulgaris var. cicla*)
pH	5.86
Nitrates, mg/kg	21600.0
Moisture	8.5
Ash	19.61
White	28.76
Fat	2.61
Food fibers	20.91
Carbohydrates	40.52

Recipe for fermented dried sausages. Five samples of fermented dried sausages were prepared (Table 2): C1 (without nitrite and sodium nitrate), C2 (100 mg/kg sodium nitrite and 100 mg/kg sodium nitrate), M1 (0.5 % chard powder), M2 (1 % chard powder) and M3 (1.5 % chard powder). Sourdough in the amount of 0.30 g/kg (*Staphylococcus xylosus+Pediococcus pentosaceus*) was added to the recipe of fermented dried sausages for samples C1, C2, M1, M2, M3. Each sample was prepared for research three times.

Physico-chemical composition and yield of fermented dried sausages. The physicochemical composition of fermented dried sausages enriched with chard powder is given in Table 3. The pH of fermented dried sausages at the beginning of drying and fermentation did not undergo significant changes when chard powder was added and was at the level of 5.51–5.56, which is 2 % less than control samples C1 and C2. During fermentation for 35 days, the pH of sample C1 decreased by 14.64 %, and sample C2 by 15.48 %, compared to the beginning of drying and fermentation. The pH decreased the most during drying for 35 days in sample M3 by 16.9 % compared to the beginning of fermentation. Nitrate content increased with the addition of chard powder from 141.1 mg/kg in sample M1 to 422.1 mg/kg in sample M3, the change was about 300 %. Nitrites were formed throughout the fermentation process and amounted to 4.72 mg/kg for sample M3 after 35 days of production, which is 4 times higher than control sample C2. After a full production cycle for 35 days, nitrates were detected only in sample C2 in the amount of 20.6 mg/kg. The best results of improving the nutritional value of fermented dried sausages, after 35 days of drying and fermentation, compared to the control (C1 and C2) were shown by samples M2 and M3, although the addition of even 0.5 % chard powder significantly affected the protein, ash and dietary fiber content. The protein content of dried fermented sausages with the addition of 1 % (Sample M2) chard powder increased by 18.61 % and 20.39 %, the dietary fiber content increased by 86.36 % and 90.91 % compared to controls C1 and C2, in accordance. The ash content in sample M2 increased by 35.03 % compared to the control. The energy value of the fermented dried sausages of Sample M2 was 424.17 kcal/100 g and was 13.7 % higher than the energy value of the control sausage samples, which is due to the lower moisture content in the samples with chard powder. The protein content in dried fermented sausages with the addition of 1.5 % (Sample M3) chard powder increased by 21.01 % and 22.74 %, the dietary fiber content increased by 90.63 % and 93.75 % compared to control C1 and C2, respectively. The ash content in sample M3 increased by 35.03 % compared to the control. The energy value of the fermented dried sausages of Sample M3 was 425.52 kcal/100 g and was 14 % higher than the energy value of the control sausage samples, which is also due to the lower moisture content of the samples with chard powder.

Table 2

Recipes of fermented dried sausages enriched with chard powder $n=3$

Formulation component, g/kg	C1	C2	M1	M2	M3
	without nitrite and sodium nitrate	100 mg/kg of nitrite and sodium nitrate	0.5 % chard powder	1 % chard powder	1.5 % chard powder
Chard powder	0	0	5	10	15
Pork	800	800	800	800	800
Pork fat	185	185	180	175	170
Glucose	10	10	10	10	10
Garlic	3	3	3	3	3
White pepper	1.2	1	1.2	1.2	1.2
Leaven	0.3	0.3	0.3	0.3	0.3
Nitrite and sodium nitrate	0	0.2	0	0	0
Sodium chloride	0.25	0.25	0.25	0.25	0.25
Sodium ascorbate	0.25	0.25	0.25	0.25	0.25

Table 3

Physico-chemical composition of fermented dried sausages enriched with chard powder $n=3$

Parameters, %	Fermented dried sausages enriched with chard powder									
	Start of drying and fermentation (raw product)					35 days of drying and fermentation (final product)				
	C1	C2	M1	M2	M3	C1	C2	M1	M2	M3
pH	5.6	5.62	5.52	5.51	5.56	4.78	4.75	4.68	4.72	4.62
Nitrates, mg/kg	0	48.6	141.1	275.6	422.1	0	20.6	0	0	0
Nitrites, mg/kg	0	92.2	0	0	0	0	1.18	0.45	2.75	4.72
Moisture	62.8	62.2	62.7	62.5	62.1	45.5	45.8	36.8	35.2	34.2
Ash	0.91	0.93	1.01	1.11	1.21	1.28	1.28	1.72	1.97	2.2
White	17.11	17.1	17.25	17.4	17.54	25.15	24.6	29.4	30.9	31.84
Fat	19.14	19.74	18.9	18.74	18.8	28.01	28.28	31.85	31.49	31.12
Food fibers	0.04	0.03	0.14	0.25	0.35	0.06	0.04	0.23	0.44	0.64

The yield and change in moisture content of fermented dried sausages enriched with chard powder are shown in Fig. 1. At the beginning of the fermentation and drying process, the moisture content of all sausage samples did not differ significantly. After fermentation and drying for 35 days, a decrease in the moisture content of finished sausages was observed with an increase in the content of chard powder in the recipe of fermented dried sausages (Table 2), which significantly affected the yield of the food product. Compared to control sample C1, the moisture content of fermented dried sausages after 35 days of fermentation and drying in samples with chard powder decreased by 23.64 % for sample M1, by 29.26 % for sample M2 and by 33.04 % for sample M3.

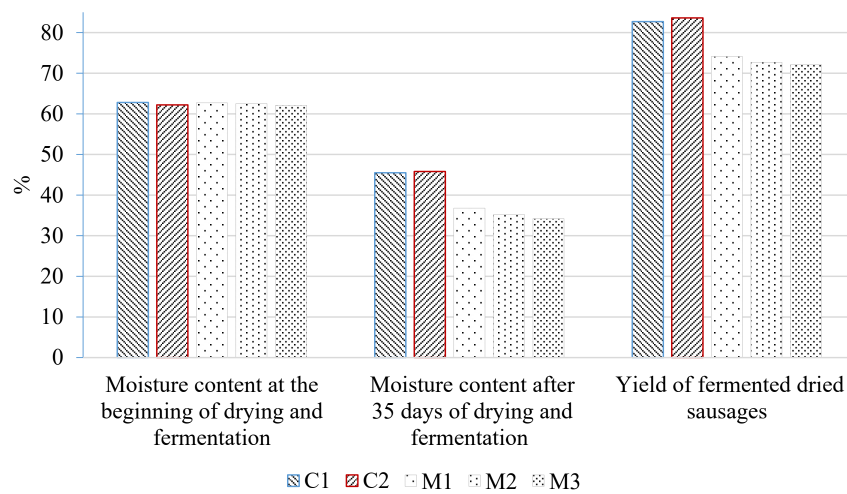

Fig. 1. Yield and change in moisture content of fermented dried sausages enriched with chard powder $n=3$

The yield of the food product also decreased compared to the C1 control by 11.61 % for the M1 sample, 13.76 % for the M2 sample, and 14.7 % for the M3 sample. Compared to control sample C2, the moisture content of fermented dried sausages after 35 days of drying and fermentation in samples with chard powder decreased by 24.46 % for sample M1, by 30.11 % for sample M2, and by 33.92 % for sample M3. The yield of the food product also decreased compared to control C2 by 12.82 % for sample M1, by 15 % for sample M2 and by 15.95 % for sample M3 (Fig. 1).

Organoleptic parameters of fermented dried sausages enriched with chard powder. Organoleptic indicators of fermented dried sausages enriched with chard powder for samples M2 and M3 were better in terms of color indicators than those of other samples, which is related to the natural color of chard and texture, which is related to a lower product yield (Fig. 1) and the presence dietary fibers (Table 3), shown in Fig. 2. The overall acceptability was the highest in sample M3 and amounted to 8.5 units, which is 0.4 units more than sample C2 and 0.7 units more than sample C1. Sample M2 also had a high total acceptance score of 8.4 units, which is 0.6 units higher than sample C1 and 0.3 units higher than sample C2.

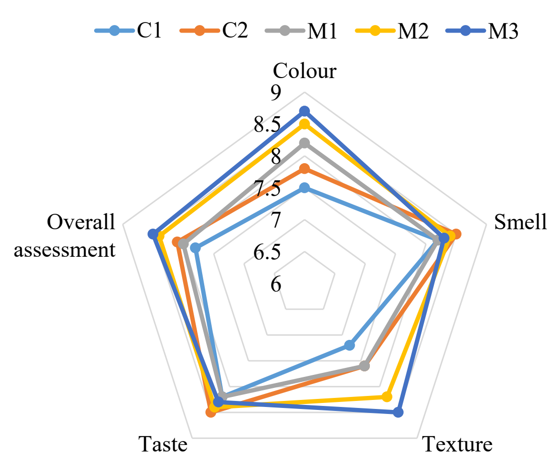

Fig. 2. Organoleptic analysis of fermented dried sausages enriched with chard powder

Microbiological analyzes of fermented dried sausages enriched with chard powder. The results of counting aerobic mesophilic and lactic acid bacteria, as well as the total number of coliform bacteria are presented in the Table 4.

Table 4

Microbiological analyzes of fermented dried sausages enriched with chard powder $n=6$

Parameters, %	Fermented dried sausages enriched with chard powder									
	Start of drying and fermentation (raw product)					35 days of drying and fermentation (final product)				
	C1	C2	M1	M2	M3	C1	C2	M1	M2	M3
Aerobic mesophilic bacteria, log CFU/g	6.75	6.82	6.98	7.06	7.11	8.85	9.67	9.15	10.22	10.27
Lactic acid bacteria, log CFU/g	5.82	6.05	6.22	6.25	6.32	9.61	9.85	9.62	10.75	10.82
Total coliforms, MPN/g	52	108	256	438	870	<3	<3	<3	<3	<3

Sourdough, which is a mixture of bacteria (*Pediococcus pentosaceus*) and taste bacteria (*Staphylococcus xylosus*), is responsible for lowering the pH, converting sugar into lactic acid and improving the physicochemical properties and sensory characteristics of fermented products. The content of lactic acid bacteria at the beginning of fermentation and drying of fermented dried sausages enriched with chard powder was at the level of 5.82 to 6.32 log CFU/g. These values will increase during ripening as the starter bacteria will grow and produce lactic acid to lower the pH and reach a lactic acid bacteria population of around 9–10 log CFU/g at the end of drying. After 35 days of treatment, final counts ranged between 9.61 and 10.82 log CFU/g for treatments C1 and M3, respectively. The content of aerobic mesophilic bacteria at the beginning of fermentation and drying of fermented dried sausages enriched with chard powder was at the level of 6.75 to 7.11 log CFU/g. The count of aerobic mesophilic bacteria was similar to the count of lactic acid bacteria, showing that these groups of microorganisms predominate in fermented dry sausages. Sourdough may be responsible for the increase of aerobic mesophilic bacteria during the ripening period, as confirmed by their increase to the level of 8.85–10.27 log CFU/g after 35 days of drying and fermentation. Despite the high initial values of total coliforms found in fermented dried sausages, especially with the addition of chard powder, which ranged from 256 to 870 MPN/g. Due to the lowering of the pH and the development of the leaven, the safety of the product was confirmed at the end of the treatment, which was at a level of less than 3 MPN/g for all the sausage samples.

Chard powder obtained by the method of microwave vacuum drying is a new raw material for fermented sausage products. Semi-finished products from chard will be suitable for use in the recipes of other food products, not only as a source of nitrites, but also for enrichment with vegetable protein, dietary fibers and minerals. chard powder has shown food coloring properties, but it is necessary to conduct research on professional equipment to determine the exact color indicators. The obtained research results were influenced by the chard variety, the equipment for microwave vacuum drying (laboratory drying) and the number of repeated experiments. Initially, the study planned to use several varieties of chard, but martial law and hostilities destroyed part of the farms that had varieties unique to Ukraine.

Limitations include the fact that the research was conducted on morally outdated domestic equipment, which, due to the state of war and reduced funding, cannot yet be updated. And this could affect the accuracy of the measurements.

Further research should be directed at different methods of drying chard, research on pigments and color indicators, qualitative analysis of mineral substances and the use of chard powder in recipes of other food products.

4. Conclusions

Chard powder obtained by microwave vacuum drying of «Burpee's Rhubarb Chard» (*Beta vulgaris var. cicla*) can be effectively used as an alternative to nitrate and nitrite in fermented dried sausages. The addition of chard powder reduced the moisture content, pH and yield of fermented dried sausages. The content of nitrites in samples with chard powder does not exceed the permissible standards and fulfills all its technological functions, such as improving the color, structure and microbiological indicators of fermented dried sausages. The main negative effect of adding chard powder to the sausage recipe was the high coliform content at the beginning of drying and fermentation, which is related to the microbiological indicators of the vegetable powder. The content of aerobic mesophilic bacteria increased by 5.84 % and the content of lactic acid bacteria increased by 8.96 % in sample M3 compared to control C2 after 35 days of fermentation and drying, which is related to the activity of the starter. After 35 days of fermentation and drying, the coliform content was normalized to safe levels thanks to the starter. Sample M3 (1.5 % chard powder) proved to be the best for replacing nitrates and nitrites in fermented dried sausages, as well as increasing the nutritional value, organoleptic and microbiological indicators of sausages. This research sets the stage for increasing the choice of natural sources of sodium nitrite, due to the high nitrate content of chard powder.

Conflict of interest

The authors declare that they have no conflict of interest in relation to this research, whether financial, personal, authorship or otherwise, that could affect the research and its results presented in this paper.

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Data availability

The manuscript has no associated data.

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