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Innovations in the meat industry: Natural colorants for organic sausages without nitrites

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Abstract

The purpose of the research was to produce sausages (hot dogs) in which the composition of the meat emulsion has been altered and to compare their sensory properties and microbiological quality. For this purpose, three different emulsions were prepared. The control emulsion (sample 1) was prepared according to the recipe for organic beef products. Beetroot powder was added to the second emulsion (sample 2). In the third emulsion (sample 3), half of the iodized sea salt's share was replaced by nitrite salt. The products were analyzed for the total number of microorganisms on the 1st, 7th, 14th and 21st days of storage. Sensory quality of produced hot dogs was also evaluated.

Keywords: meat emulsion, iodized sea salt, beetroot, sodium nitrite, sensory evaluation, microbiological analysis

Introduction

The needs and demands of consumers in the market are increasing day by day. People have a wide range of foods to choose from, including meat and meat products. The trend towards healthy eating is growing, and as a result, we strive to adhere to modern guidelines for a healthy diet when producing meat products.

Some people include meat and meat products in their diet almost daily. For many, meat serves as a source of energy and necessary protein intake. Meat is rich in micronutrients, especially B-complex vitamins and vitamin A. Among minerals, it contains significant amounts of iron, phosphorus, zinc, magnesium, and potassium. These micronutrients are crucial for the normal functioning of our bodies. Meat is composed of various nutrients and water, making it an ideal medium for the growth of microorganisms. To extend the shelf life and reduce the growth of naturally present microorganisms, various preservation methods are employed.

Preservation is mostly carried out during processing by adding additives. Therefore, the production of preserved meat and meat products involves heat treatment and the addition of additives. Today, the most used additives are nitrates, nitrites, and polyphosphates. However, due to research showing that excessive doses of these substances can be harmful to human health, their use is regulated by law.

Nitrates and nitrites are used as preservatives in meat products to prevent the growth of bacteria and foodborne diseases, including botu-

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lism caused by the bacterium *Clostridium botulinum*, as coloring agents (imparting a red or pink color to the product), and as flavour enhancers. In the production of heat-treated products, sodium nitrate (E251), potassium nitrate (E252), sodium nitrite (E250), and potassium nitrite (E249) are used the most. The issue with using nitrite in production of meat products lies in its carcinogenicity, particularly if consumed in excessive amounts. It was proven that nitrite present in foods can form genotoxic and carcinogenic N-nitroso compounds in the human body. However, not all compounds are carcinogenic or genotoxic (Filipič, 2006).

Beetroot belongs to the root vegetable family, and its tubers can be either round or elongated in shape. The main root can grow up to one meter in length, while most of the roots develop at a depth of up to 40 cm (Cisem et al., 2018). Despite its name, beetroot can also be differently colored, with tubers mostly reddish, but also yellow. The lighter the leaves, the lighter the tuber. The tuber can be consumed fresh, cooked, baked, or pickled as well. Young leaves can be prepared in the same way as spinach. Beetroot contains beneficial anthocyanins, which act as antioxidants (Honikel, 2008). Compared to other types of vegetables, beetroot has a higher carbohydrate content and is also very rich in sugars. In the food industry, beetroot is used as a food coloring, dietary fiber, or antioxidant. To develop and maintain the pink color of boiled sausages, beetroot coloring (betanin) is added at the beginning of the emulsifying process (Aykın-Dinçer et al., 2021). This gives the products a quick, strong, and stable color. Beetroot juice is an alternative to synthetic pigments, which can have numerous negative effects on human health. Long-term consumption can cause allergic reactions, and some can even cause cancer. Beetroot is primarily found in food and beverages. It can be dehydrated in the form of powder or flakes. Beetroot juice can be used to enhance the color of tomato pastes, soups, sauces, desserts, jams, jellies, candies, and breakfast cereals (Podgoršek, 2010).

Beetroot pigments, known as betalains, can be divided into two groups: red betacyanins and yellow betaxanthins. Betalains are highly water-soluble (Valencia et al., 2020). Betanin is the red betacyanin responsible for 75 to 90 % of the color in beetroot. As a food additive, it is labeled with the number E 162 (Hendry et al., 1996).

The aim of the research was to find a natural colorant for the meat emulsion of organic beef hot dogs and to determine how the selected colorant affects the sensory properties. We aim to choose a natural additive that does not contain nitrite and has a color effect at least approximately similar to that of nitrite. Colorants are added to foods to mask their true color, making dishes more attractive and enticing customers to purchase the products.

Materials and methods

Materials

To produce organic hot dogs, we used the following organic ingredients:

- Organic beef from categories II and III,
- Organic spices produced by the company Raps,
- Organic sunflower oil from Cekin,
- Beetroot powder from Umami Foods,
- Iodized sea salt from Mercator.

Hot dogs are produced according to three different recipes: basic organic recipe, with addition of beetroot (beetroot powder), and with addition of nitrate salt.

Sample 1 was an organic beef hot dog made according to the basic recipe. It was composed of organic beef from category II and III, ice, organic sunflower oil, added spices, and iodized sea salt (1.5 %). In Sample 2, beetroot powder was added to the basic recipe (0.05 %). In the third sample, the same recipe was used as in the first sample, but half of the iodized sea salt was replaced with nitrite salt, and Sample 3 contained half of the iodized sea salt (0.75 %) which was replaced with 0.75 % of nitrite salt.

Appearance of hot dog fillings is presented in Fig. 1.

Technological process of hot dog production

The hot dogs were produced under industrial manufacturing conditions. Each sample was made in quantities of 5 kg. For the technological process of hot dog production, we used the following equipment:

- Mado grinder, Ultra2
- Mado cutter, Supercutter
- REX RVF 55 filler
- Fessmann thermal chamber
- Ice maker
- Fessmann shower

The meat must be well chilled before it is

Fig. 1. Appearance of Hot dog fillings (Sample 1 contains iodized sea salt, sample 2 contains beetroot powder, and sample 3 contains iodized sea salt and nitrite salt).

placed in the grinder and ground through a plate with a diameter of 3 mm. The ground meat is weighed and placed in the cutter. The meat is chopped with the addition of spices, salt, vegetable oil and ice. The mixture is stuffed into sheep casings with a diameter of 24-26 mm and formed into pairs, weighing between 120 and 150 grams each. The hot dog pairs are hung on aluminium rods and neatly arranged so that they do not touch each other. Before thermal processing, they are showered to remove any foreign particles. This is followed by heat treatment, during which it is important to reach an internal temperature of +72 °C. After completing the thermal process, sausages are moved to a cooling room with a temperature of 2 °C. The next day, they are vacuum-packed and stored in the cooling room. We stored the hot dogs in the refrigerated conditions at the same temperature of 2 °C for 3 weeks. One pair of hot dog samples had a mass of 150 g. A total of 33 pairs of individual hot dog samples were produced. By "sample of individual hot dogs," a pair of hot dogs is meant.

Methods

Determination of total microorganism count (TMC)

The microbiological examination included the determination of the total microorganism count (TMC). We conducted a microbiological analysis at The Education Center Piramida Maribor.

We analysed all three pairs of hot dog samples (sample 1, sample 2 and sample 3) on the first, seventh, fourteenth, and twenty-first day from the day of production. For microbiological analyses, we used the Petrifilm counting method, performed according to the prescribed standard protocols SIST ISO 4833-1:2013 (https://www.iso.org/standard/53728.html)

Preparation of serial decimal dilutions of hot dog samples

All microbiological analyses of the hot dog samples were conducted under aseptic conditions. After homogenization of the hot dog samples, we prepared four decimal dilutions up to a 10-4 to ensure that the nutrient agar plates were countable.

Method for determining the total microorganisms count

From the appropriate dilutions, we pipetted 1 mL of the sample and transferred it onto the Petrifilms that were incubated for 24 hours at 37 °C. After 24 hours, we counted all the colonies that grew on the medium, considering the dilutions. The results are expressed as the number of microorganisms per g of sample.

Calculation of colony-forming units

After incubation, we counted the grown colonies according to Nahberger Marčič (2008). Countable Petrifilms are those with 10 to 300 colonies. The number of colony-forming units per mL of sample was calculated using the equation:
Where:

$$N = \frac{\sum C}{(n_1 + 0{,}1 * n_2)d}$$

- ∑C – the sum of all colonies on all Petrifilms
- n1 – number of colonies at the first dilution
- n2 – number of colonies at the second dilution
- d – dilution factor at the first considered dilution
- N – number of microorganisms in CFU/g/mL (Nahberger Marčič, 2008)

Sensory analysis

For sensory evaluation, we used the trade fair method for evaluating meat products. This method is based on a system of deducting points for the following attributes of hot dogs: external appearance, cross-sectional composition, cross-sectional color, texture, smell, and taste. During the point deduction, the evaluation committee provided notes for each attribute on the evaluation sheet. The hot dog samples were thermally processed. After processing, the hot dogs were cut on boards and the samples were distributed to the committee members.

Trade fair method for evaluating hot dogs:
Appearance (0 – 2 points)
Evaluation of the product's external appearance.
2 – characteristic, excellent sensory impression, appropriate shape and color,
0 – irregular shape, wrinkled casing, damaged casing, inappropriate color.
Cross-sectional composition (0 – 3 points)
Evaluation of the cross-sectional appearance of the hot dog filling (cold, warm), along the longitudinal section of the half.
3 – uniform cross-section without holes,
0 – coarse, holey structure, uneven distribution of particles.
Cross-sectional color (0 – 3 points)
Evaluation of the uniformity of the cross-sectional color.
3 – uniform typical color,
0 – uneven color, discoloration, too dark, too light color.
Texture (0 – 4 points)
4 – the filling is chewy and smooth,
0 – the filling is grainy, sandy, crumbly, rough, greasy, rubbery, soft.
Smell (0 – 3 points)
3 – typical, well-expressed smell,
0 – putrid smell, sour, foreign smells, very poorly expressed typical smell.
Taste (0 – 5 points)
5 – pleasant, characteristic of hot dogs,
0 – burnt, sour, salty, one-sided seasoning, tasteless.

Results and discussion

Results of determining total microorganism count

Results of microbiological analysis are presented in Table 1.

Microbiological tests were conducted on the first day when the hot dogs were produced. Subsequent analyses were performed after 7, 14, and 21 days of storage. In Sample 1, where only iodized sea salt was added, the highest number of microorganisms is expected. TMC increases during storage in all dilutions and samples. In Sample 2, where beetroot powder was added in addition to iodized sea salt, it is observed that the number of microorganisms does not significantly differ after 7 and 14 days of storage. The number of microorganisms slightly increased but remained at a low level. In sample 3, where nitrite salt was added, the number of microorganisms did not change after 7, 14, and 21 days of storage. The number of colonies significantly increased after 21 days of storage.

The results of the analysis for the Sample 2 with added beetroot powder showed a similar microbiological profile to Sample 3, for which the hot dogs were made with added nitrite. In Sample 3, in which nitrite salt was added, the effect of the nitrite salt was evident on all sampling dates. A noticeable difference was observed especially during the final sampling at 21st day of storage.

Results of sensory analysis

The sensory evaluation was conducted by five experienced experts. The hot dogs were thermally processed and then evaluated using a fair assessment form, with notes provided for each

Table 1 Results of determining of colony count in hot dog samples during storage

Samples*	Dilutions	Total microorganisms count, CFU/g			
		24 h	7 days	14 days	21 days
Sample 1	10^{-1}	42	innumerable	innumerable	innumerable
	10^{-2}	6	30	210	innumerable
	10^{-3}	0	0	44	innumerable
	10^{-4}	0	0	11	290
Sample 2	10^{-1}	73	79	innumerable	innumerable
	10^{-2}	6	7	260	innumerable
	10^{-3}	0	2	33	innumerable
	10^{-4}	0	0	2	250
Sample 3	10^{-1}	31	innumerable	innumerable	innumerable
	10^{-2}	3	17	94	innumerable
	10^{-3}	0	1	4	230
	10^{-4}	0	0	0	46

** Sample 1 – basic organic recipe; Sample 2 – addition of beetroot powder; Sample 3 – addition of nitrate salt*

characteristic. The following attributes were assessed: external appearance, cross-sectional composition, cross-sectional color, texture, aroma, and taste are presented in Fig. 2.

Sample 1 (organic hot dog) received points deducted from all evaluators. The evaluators wrote

Fig. 2. External appearance and cross sectional composition of hot dogs. (Sample 1 contains iodized sea salt, sample 2 contains beetroot powder, and sample 3 contains iodized sea salt and nitrite salt).

the following comments under the notes: pale without typical color and visible small air bubbles. Sample 2 (organic hot dog with added beetroot) also received deducted points. Comments followed: too intense color, visible air bubbles. The result of these notes is due to an excessive amount of beetroot. Sample 3 (organic hot dog with nitrite) received the least points, as the color of the hot dogs was suitable. When compiling the cross-section, all evaluators deducted points. For all three samples, pitting notes were noted. The cross-section color of sample 1 was pale and uneven, in some places greyish. Sample 2 was too intensely red. In sample 3, greyish tints occasionally appeared. The texture was decent and received very few bounce points. The smell was rated very well in all three samples. The samples had a pleasant, harmonious smell. The members of the evaluation committee were disturbed by the hot dog samples' taste being too salty and hot. The samples were made on a vacuum filler and therefore there were not so many bounce points. In sample 2, we reduced the amount of beetroot. Again, sample 1 received de-points in external appearance and cross-sectional color due to pale color. The other two samples had a nice typical color. When compiling the cross-section, the following notes appeared in all samples: slight and larger pitting, and a rough cross-section. In terms of texture, reviewers found sample 1 to be too hard and a bit rubbery. The expert panel evaluated the smell parameter without deducting points, but for sample 3, the evaluators deducted points due to the smell of the brine. A spicy taste was detected in all samples according to the testers' assessment.

Based on the obtained results, we can conclude that the addition of beetroot powder positively affects the color stability, achieving approximately the same shade as in Sample 3, where nitrite was added. Additionally, we can assert that beetroot powder in Sample 2 provided the same shelf life of three weeks as for the hot dogs with added nitrite. Meanwhile, in Sample 1, in which only sea salt was added, we did not achieve satisfactory color for the consumer, and the product's shelf life was only 14 days. In Sample 1, a sour aftertaste was detectable after 14 days. This taste was only noticeable after 21 days in Samples 2 and 3.

Conclusions

With the results of sensory evaluations, we proved that with the correct amount of beetroot powder, we succeeded in improving the color of the hot dogs compared to organic hot dogs without the addition of beetroot.

Results of microbiological analysis confirmed that beetroot powder has a potential antimicrobial effect. In the first 14 days, the results are even comparable to Sample 3 with addition od nitrate salts. However, on the 21st day of storage, the number of microorganisms increased quite a bit.

Based on sensory analysis and microbiological analysis, we can conclude the following: beetroot powder as a natural colorant, can be used as effective replacement for nitrites in meat products, however, there is still no single replacement for nitrite that summarizes all the functions it has in the production of meat products.

References

[1] **Filipič, M. (2006):** Genotoksične komponente hrane. V: Karcinogene in antikarcinogene komponente v živilih. 24. Bitenčevi živilski dnevi, 9. in 10. november 2006, Ljubljana, Biotehniška fakulteta, Oddelek za živilstvo: 61-72.

[2] **Podgoršek, J. (2010):** Rdeča pesa – znova na naših njivah? Glas dežele. Dostopno na: https://dk.um.si/Dokument.php?id=19630&lang=slv [Uporabljeno 7. 10. 2022].

[3] **Hendry, G.A.F., Houghton, J.D. (1996):** Natural food colorants. 2nd ed. Glasgow: Blackie Academic & Professional, Chapman & Hall, str. 348 – 349

[4] **ISO 4833-1:2013. (2019):** Microbiology of the food chain — Horizontal method for the enumeration of microorganisms https://www.iso.org/standard/53728.html

[5] **Nahberger Marčič, V. (2008):** Živilska mikrobiologija in biotehnologija. Vaje iz mikrobiologije. Ljubljana: Izobraževalni center Piramida Maribor, Višja strokovna šola.

[6] **Aykın-Dinçer, E., Güngör, K., Çağlar, E. and Erbaş, M. (2021):** The use of beetroot extract and extract powder in sausages as natural food colorant. International Journal of Food Engineering, Vol. 17 (Issue 1), pp. 75-82. https://doi.org/10.1515/ijfe-2019-0052

[7] **Cisem, S., Gulen Yildiz T. (2018):** The investigation of the use of beetroot powder in Turkish fermented beef sausage (sucuk) as nitrite alternative, Meat Science,Volume 140, Pages 158-166, https://doi.org/10.1016/j.meatsci.2018.03.012

[8] **Honikel K. O. (2008):** The use and control of nitrate and nitrite for the processing of meat products, Meat Science, Volume 78, Issues 1–2, Pages 68-76, https://doi.org/10.1016/j.meatsci.2007.05.030

[9] **Valencia, R. D., Munekata, P. E., Pateiro, M., Maggiolino, A. (2020):** Red Beetroot. A Potential Source of Natural Additives for the Meat Industry. Applied Sciences. 10. 8340. DOI:10.3390/app10238340

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Inovacije u mesnoj industriji: prirodna bojila za organske kobasice bez nitrita

Sažetak

Cilj istraživanja bio je proizvesti hrenovke (hot dog) u kojima je izmijenjen sastav mesne emluzije, te usporediti njihova senzorna svojstva i mikrobiološku kvalitetu. U tu su svrhu pripremljene tri različite

emulzije. Kontrolna emulzija (uzorak 1), pripremljena je prema receptu za organske proizvode od govedine. U drugu emulziju (uzorak 2) dodana je cikla u prahu. U trećoj emulziji (uzorak 3) pola udjela jodirane morske soli zamijenjeno je s nitritnom soli. Proizvodi su analizirani na ukupni broj mikroorganizama 1., 7., 14. i 21. dana pohrane. Također, ocijenjena je senzorna kvaliteta proizvedenih hrenovki.
Keywords: mesna emulzija, jodirana morska sol, cikla, natrijev nitrit, senzorna evaluacija, mikrobiološka analiza

Innovationen in der Fleischindustrie: Natürliche Farbstoffe für Bio-Würste ohne Nitrite

Zusammenfassung

Ziel der Untersuchung war es, Würste (Hot Dogs) herzustellen, bei denen die Zusammensetzung der Fleischemulsion verändert wurde, sowie ihre sensorischen Eigenschaften und mikrobiologische Qualität zu vergleichen. Zu diesem Zweck wurden drei verschiedene Emulsionen hergestellt. Die Kontrollemulsion (Probe 1) wurde nach der Rezeptur für Bio-Rindfleischprodukte zubereitet. Der zweiten Emulsion (Probe 2) wurde Rote-Bete-Pulver zugesetzt. In der dritten Emulsion (Probe 3) wurde die Hälfte des Anteils an jodiertem Meersalz durch Nitritsalz ersetzt. Die Produkte wurden am 1., 7., 14. und 21. Tag der Lagerung auf die Gesamtzahl der Mikroorganismen untersucht. Die sensorische Qualität der hergestellten Hot Dogs wurde ebenfalls bewertet.
Schlüsselwörter: Fleischemulsion, jodiertes Meersalz, Rote Bete, Natriumnitrit, sensorische Bewertung, mikrobiologische Analyse

Innovaciones en la industria cárnica: Colorantes naturales para salchichas orgánicas sin nitritos

Resumen

El propósito de la investigación fue producir salchichas (ing. hot dogs) en las que se ha alterado la composición de la emulsión cárnica y comparar sus propiedades sensoriales y calidad microbiológica. Para este propósito, se prepararon tres emulsiones diferentes. La emulsión de control (muestra 1) fue preparada según la receta para productos de carne de res orgánica. Se añadió polvo de remolacha a la segunda emulsión (muestra 2). En la tercera emulsión (muestra 3), se reemplazó la mitad de la sal marina yodada por sal con nitrito. Los productos se analizaron para determinar el número total de microorganismos en los días 1, 7, 14 y 21 de almacenamiento. También se evaluó la calidad sensorial de las salchichas hot dogs producidas.
Palabras claves: emulsión cárnica, sal marina yodada, remolacha, nitrito de sodio, evaluación sensorial, análisis microbiológico

Innovazioni nell'industria della carne: coloranti naturali per insaccati biologici senza nitriti

Riassunto

L'obiettivo della ricerca era produrre wurstel da hot dog in cui fosse cambiata la composizione della carne emulsionata e confrontare le loro proprietà sensoriali e la qualità microbiologica. A questo scopo sono state preparate tre diverse emulsioni. L'emulsione di controllo (campione 1) è stata preparata secondo la ricetta per i prodotti a base di carne bovina biologica. Alla seconda emulsione (campione 2) è stata aggiunta polvere di barbabietola rossa. Nella terza emulsione (campione 3), metà del sale marino iodato è stato sostituito con nitrito di sodio. I prodotti sono stati analizzati per il numero totale di microrganismi al 1°, 7°, 14° e 21° giorno di conservazione. Inoltre, è stata valutata la qualità sensoriale dei wurstel prodotti.
Parole chiave: carne emulsionata, sale marino iodato, barbabietola, nitrito di sodio, valutazione sensoriale, analisi microbiologica