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Application of spruce needle extracts in meat analogue development

Darius Černauskas, Kaunas University of Technology

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OBJECTS

Spruces needles

Meat analogues

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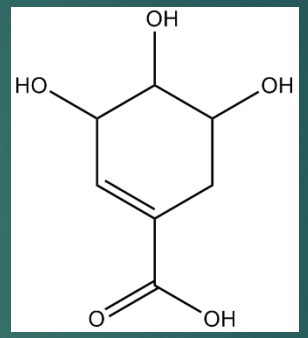
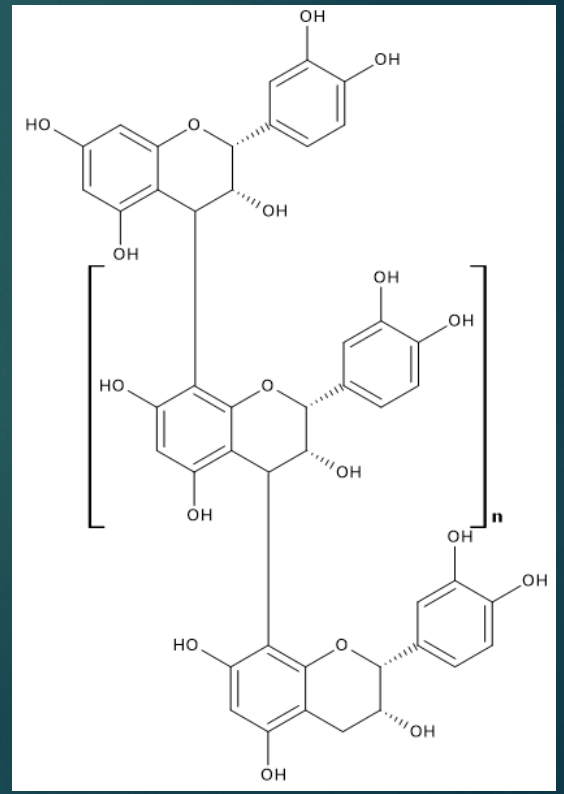
Spruces needles



Shikimic acid

Proanthocyanidins



OBJECTS

Ingredients: water, isolated SOY protein, corn starch, preservative: buffered vinegar (E267); dextrose, thickeners: carrageenan (E407), konjac gum (E425), locust bean gum (E410); firming agent: potassium chloride (E508); red radish concentrate, coloring: betanin (E162), refined sunflower oil, pea fiber.

Meat analogues

METHODS

Chemical extraction of spruce needles from KTU

Preparation of spruce needles :
WASHING

14-year-old spruce (*Picea pungens*) needles were selected for extraction. The needles were collected from the branches of a felled tree. Branches with needles were kept outside (June-February) until the washing and crushing operations. The cut spruce branches were washed with tap water and left to dry at room temperature (20 °C) 12 h. 5 liters of tap water were used to obtain 100 g of needles. Later, the needles are manually separated from the branches. After preparation, needles were stored at room temperature in sealed containers at an ambient humidity of 43%.

Preparation of spruce needles:
CUTTING

The needles were 1.6 ± 0.2 cm long before crushing. Washed spruce needles 100 g, crushed in 10 series using laboratory IKA Batch Cutting/Impact Mills. 1 session lasts 3 min, speed 25000 rpm. The resulting biomass is dried for 24 hours at room temperature. Later, the procedure is repeated. Powder of needles with a moisture content of 1.4 % is obtained. The determined particle size of ground needles varied d10 = 14,3 µm, d50 = 101 µm, and d90 = 516 µm means that 10% of the sample is smaller than 14,3 µm, 50% is smaller than 101 µm, and 90% is smaller than 516 µm.

Preparation of spruce needles extract
EXTRACTION

Powder of spruce needles 100 g mixed with 2000 mL of distilled water. The solution boiled at 100 °C in flat bottom flask with joint using coiled condenser, for 5 h. During the extraction, the mixture was additionally stirred at a speed of 300 rpm.

Preparation of spruce needles extract
COOLING AND SEPARATION

The solution cooled down at room temperature (20-22°C), 30 min. First, the solution is filtered through a paper filter. This way needles separates the from the extract. Later, the extract is filtered through a glass filter filled with zeolite. In this way, water-insoluble components are separated.

A vacuum pump and Bunsen flask were used. A paper filter was placed in a Buchner funnel. Characteristics of zeolite:

1. Centrifuged Wet Density (gm/cc) 0.33
2. Water soluble substances, % 0.01
3. Filter paper: pore size 11 µm

Spruce needles extract characterization

1) The determined particle size of spruce needles extract varied d10 = 0,976 µm, d50 = 11,6 µm, and d90 = 29,3 µm means that 10% of the sample is smaller than 0,976 µm, 50% is smaller than 11,6 µm, and 90% is smaller than 29,3 µm;
2) The extract is liquid, yellowish in color. Turbidity A500 = 1.27, the extract is cloudy;
3) Proanthocyanidins was determined by the Bate-Smith assay;
4) Total phenolic concentration was analysed using the Folin–Ciocalteu assay;
5) Shikimic acid was determined by HPLC method (the extract obtained during the study was diluted 20 times with water intended for HPLC analysis. The dilution is estimated during the calculation);
6) Dry matter content in the extract 1.6%.

Extract
PACKING and STORAGE

All products (spruce needles powder and extract after extraction) are stored at -20 °C in plastic containers and bags.

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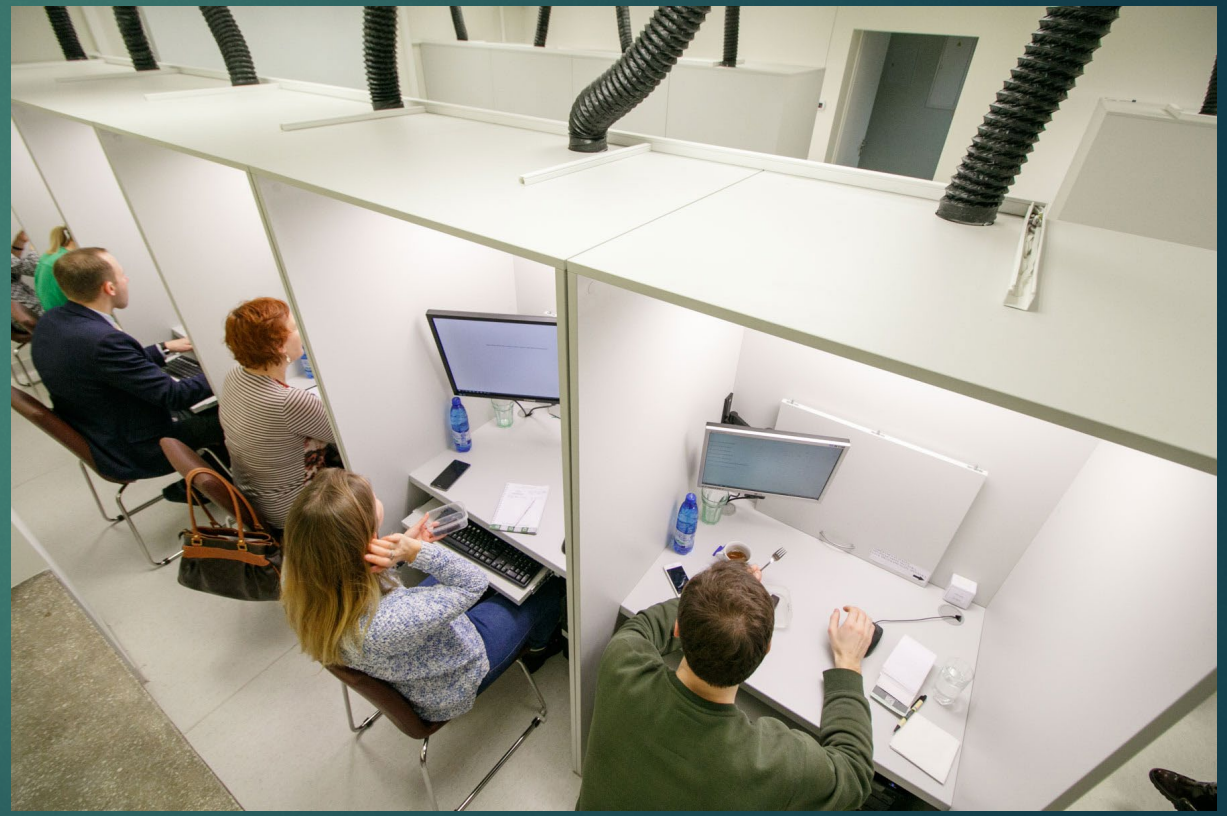
METHODS

Sensory analysis

A quantitative descriptive analysis using a 10-point scale was conducted by a 12-member sensory panel to evaluate the effect of different spruce needles extract concentrations on meat analogue matrices. Sensory attributes such as appearance, aroma, taste, texture, and aftertaste were scored from 1 (not intensive) to 10 (very intensive). Statistical analysis identified the optimal extract concentration for incorporation.



METHODS

Microbiology analysis

Microbiological analysis was conducted to evaluate the microbiological changes occurring in meat analogues during storage. The study included:

- **Total microorganism count** (LST EN ISO 4833–1:2013).
- **β-glucuronidase-producing *Escherichia coli* count** (LST ISO 16649–2:2002).
- **Coagulase-producing Staphylococcus (*S. aureus* and other species) count at 37°C** (LST EN ISO 6888-1).
- **Presumptive *Bacillus cereus* count at 30°C** (LST EN ISO 7932).
- **Detection of *Listeria monocytogenes*** (LST EN ISO 11290–1:2017).
- **Salmonella (*Salmonella* spp.) detection in 25 g (**LST EN ISO 6579-1).
- **Yeasts and molds count** (LST ISO 21527-1).

The analysis results provided insights into the safety and quality of the products during storage.

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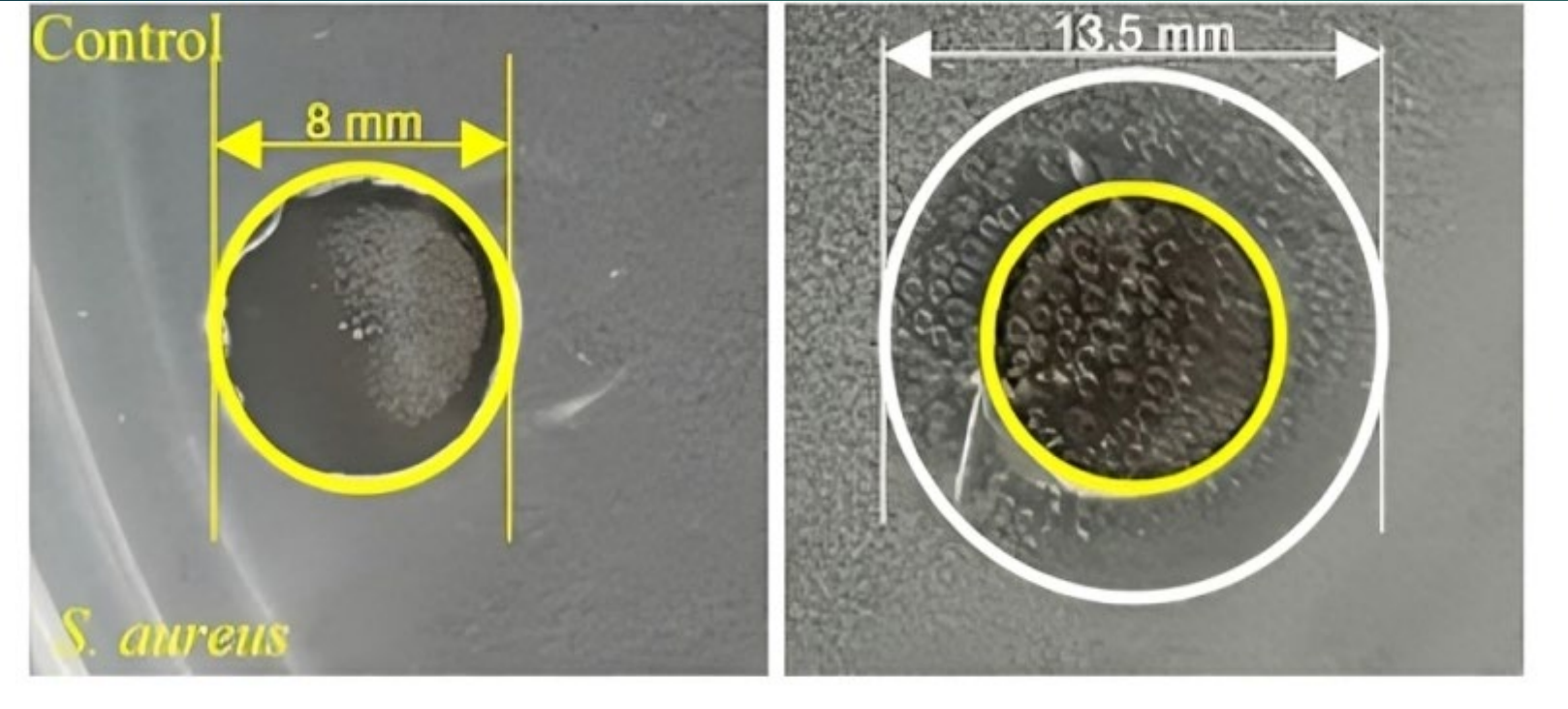
RESULTS

Microbiological characteristics of spruce needle extract



The extract obtained under optimal conditions was used in tests of antibacterial activity against food pathogens like *Listeria monocytogenes, Escherichia coli, Salmonella*, *Bacillus cereus and Staphylococcus aureus* (Fig.2). Identified concentrations showed sufficient antibacterial activity against some of pathogenic microorganism.

Fig. 2. Inhibition zones images of spruce needles extracts against *Staphylococcus aureus*

RESULTS

Piloting and evaluation of spruce needle extract in meat analogue production

Chemical extract of spruce needles

Quality and safety control:

- Microbiology analysis;
- Chemical analysis;

Meat analogues

Meat analogues with bioactive compounds

Quality and safety control:

- Sensory analysis;
- Microbiology analysis;

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RESULTS

Preparation of meat analogues with bioactive compounds for sensory analysis

Meat analogues with bioactive compounds before cooking

Meat analogues with bioactive compounds after cooking (200 °C, 15 min)

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RESULTS

Sensory characteristics of meat analogues with bioactive compounds

Parameter	Control	0.5%	1.0%	3.0%	5.0%
Appearance	6.4 ± 1.6	6.2 ± 1.8	6.5 ± 1.5	6.8 ± 1.2	6.8 ± 1.2
Aroma	6.8 ± 1.7	7.1 ± 1.2	7.0 ± 1.5	7.3 ± 1.0	7.2 ± 1.1
Taste	6.3 ± 1.8	6.5 ± 1.5	6.4 ± 1.5	6.8 ± 1.0	7.0 ± 1.0
Aftertaste	6.3 ± 1.8	6.2 ± 1.6	6.4 ± 1.4	6.5 ± 1.3	7.0 ± 1.1
Texture	5.6 ± 1.7	6.3 ± 1.3	6.1 ± 1.5	6.6 ± 1.2	7.0 ± 1.1
Overall Impression	6.3 ± 1.6	6.5 ± 1.5	6.6 ± 1.4	6.8 ± 1.2	6.8 ± 1.2

The results of the one-way ANOVA for each parameter indicate the following:

- **Appearance**: F-statistic = 0.39, p-value = 0.82 (not statistically significant)
- **Aroma**: F-statistic = 0.29, p-value = 0.89 (not statistically significant)
- **Taste**: F-statistic = 0.39, p-value = 0.82 (not statistically significant)
- **Texture**: F-statistic = 1.20, p-value = 0.32 (not statistically significant)
- **Aftertaste**: F-statistic = 0.49, p-value = 0.74 (not statistically significant)
- **Overall Impression**: F-statistic = 0.30, p-value = 0.88 (not statistically significant)

None of the parameters show statistically significant differences (p-values > 0.05). This suggests that the tested concentrations do not have a significant impact on the sensory attributes evaluated.

RESULTS

Sensory characteristics of meat analogues with bioactive compounds

Although no statistically significant differences were found, higher concentrations (3% and 5%) generally provided better sensory ratings. Based on these trends, the 5% concentration was chosen for further study to explore its potential effects on product quality and applications in meat analogues.

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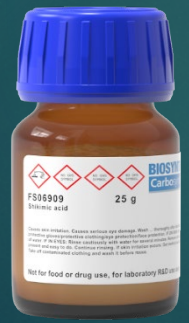
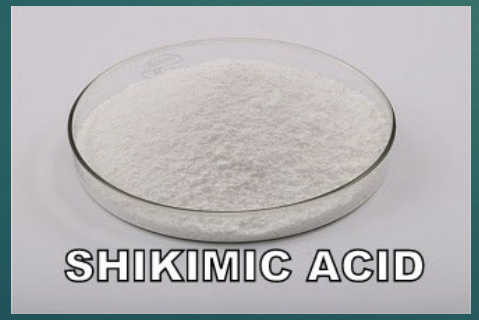
PILOTING AND EVALUATING SOLUTIONS

Schematic of the experiment

Control (0,0 %) (K)

Chemical extract of spruce needles (5,0 %) (C)





Shikimic acid powder (1,0 %) (Š)

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PILOTING AND EVALUATING SOLUTIONS

Preparation of meat analogues with bioactive for shelf life (1-7 weeks, 3 °C)

Piloting and evaluating solutions

Microbiology characteristics of meat analogues with bioactive compounds

NOT DETECTED
β-glucuronidase-producing *Escherichia coli* count, CFU/g
Coagulase-producing *Staphylococcus* (*S. aureus* and other species) count at 37°C, CFU/g
Presumptive *Bacillus cereus* count at 30°C, CFU/g
Monocytogenic Listeria (*Listeria monocytogenes*) detection at 37°C in 25 g, detected/not detected
Salmonella (*Salmonella* spp.) detection in 25 g, detected/not detected
Mold count, CFU/g

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Piloting and evaluating solutions

Microbiology characteristics of meat analogues with bioactive and natural antimicrobial compounds

Microorganism count, CFU/g

Storage Time/Parameter	Control	Chemical extract (5 %)	Shikimic acid powder (1,0 %)
After 0 week	$1,2\times10^{4}$	---	---
After 1 week	$1,3\times10^{3}$	$2,4\times10^{3}$	$1,8\times10^{3}$
After 2 week	$3,2\times10^{4}$	$1,2\times10^{5}$	$5,8\times10^{2}$
After 3 week	$1,3\times10^{6}$	$1,2\times10^{6}$	$1,4\times10^{6}$
After 4 week	$4,3\times10^{5}$	$3,6\times10^{6}$	$4,6\times10^{4}$
After 5 week	$9,1\times10^{4}$	$1,5\times10^{5}$	$4,1\times10^{4}$
After 6 week	$1,8\times10^{4}$	$8,9\times10^{4}$	$2,1\times10^{5}$
After 7 week	$2,0\times10^{4}$	$3,3\times10^{3}$	$3,6\times10^{5}$

Piloting and evaluating solutions

Microbiology characteristics of meat analogues with bioactive compounds

Yeast count, CFU/g

Storage Time/Parameter	Control	Chemical extract (5 %)	Shikimic acid powder (1,0 %)
After 0 week	$<1{,}0\times10^{1}$	---	---
After 1 week	$<1{,}0\times10^{1}$	$<1{,}0\times10^{1}$	$<1{,}0\times10^{1}$
After 2 week	$2{,}1\times10^{3}$	$4{,}1\times10^{3}$	$<1{,}0\times10^{1}$
After 3 week	$4{,}6\times10^{3}$	$6{,}8\times10^{4}$	$5{,}4\times10^{3}$
After 4 week	$7{,}4\times10^{4}$	$3{,}7\times10^{4}$	$2{,}1\times10^{4}$
After 5 week	$8{,}4\times10^{4}$	$1{,}2\times10^{5}$	$7{,}2\times10^{4}$
After 6 week	$<1{,}0\times10^{1}$	$2{,}4\times10^{4}$	$2{,}8\times10^{5}$
After 7 week	$1{,}7\times10^{3}$	$3{,}2\times10^{2}$	$1{,}1\times10^{5}$

Conclusion

Microbiology characteristics of meat analogues with bioactive compounds

Shikimic acid was initially effective in inhibiting microbial growth, but its stability decreased over time, reducing its long-term efficacy. Spruce needle extracts demonstrated protective effects against pathogenic bacteria, although their impact on overall microbial growth was limited. At the same time, incorporating these extracts into meat analogues not only enriched the products with bioactive compounds, particularly phenolic compounds, but also improved their nutritional profile and potential health benefits.

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Q&A in the chat